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# THE ANATOMICAL RECORD

EDITOR  
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VOLUME 23  
JANUARY—JULY  
1922

PHILADELPHIA  
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

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PROCEEDINGS OF THE AMERICAN ASSOCIATION  
OF ANATOMISTS  
THIRTY-EIGHTH SESSION

*Osborn Zoological Laboratory, Yale University, New Haven,  
Connecticut, December 28, 29, and 30, 1921*

WEDNESDAY, DECEMBER 28, 9.30 A.M.

The Thirty-eighth Session of the American Association of Anatomists was called to order by President CHARLES F. W. McCLURE, who appointed the following committees:

*Committee on Nominations for 1922:* Professor Alexander S. Begg, Chairman, and Professors H. B. Ferris and C. H. Danforth.

*Auditing Committee:* Professor Richard E. Scammon, Chairman, and Professor Davenport Hooker.

The remaining morning session was devoted to the presentation of scientific papers.

THURSDAY, 11:30 A.M. ASSOCIATION BUSINESS MEETING. Vice-President T. WINGATE TODD presiding.

The Secretary reported that the minutes of the Thirty-seventh Session were printed in full in *The Anatomical Record*, volume 21, number 1, pages 35 to 41. On motion, seconded and carried, the minutes of the Thirty-seventh Session were approved by the Association as printed in *The Anatomical Record*.

Professor R. E. Scammon reported for the Auditing Committee as follows: The undersigned Auditing Committee has examined the accounts of Doctor Charles R. Stockard, Secretary-Treasurer of the Association of Anatomists, and finds the same to be correct with proper vouchers for expenditures and bank balance on December 20, 1921, of \$172.86.

[Signed] R. E. SCAMMON,  
DAVENPORT HOOKER.

The Treasurer made the following report for the year 1921:

Balance on hand December 29, 1920, when accounts were last audited.....	\$164.40	
Receipts from dues 1921.....	2,881.86	
	<hr/>	
Total deposits.....		\$3,046.26
Expenditures for 1921:		
Expenses Secretary-Treasurer, Philadelphia Meeting.....	\$19.10	
Postage and telegrams.....	50.46	
Printing and stationery.....	86.74	
Collection and exchange on drafts.....	4.10	
Stenography, typewriting.....	49.50	
Paid bank for credit North Dakota check.....	7.00	
Member Second International Eugenics Congress.....	5.00	
Wistar Institute, subscriptions to Journal of Anatomy, Anatomical Record, etc.....	2,651.50	
	<hr/>	
Total expenditures.....		2,873.40
		<hr/>
Balance on hand.....		\$172.86
Balance on hand deposited in the name of the American Association of Anatomists in the Corn Exchange Bank, New York City.		

On motion, the report of the Auditing Committee and the Treasurer were accepted and adopted.

The Committee on Nominations, through its Chairman, Professor Ross G. Harrison, placed before the Association the following nominations for office in the Association: For President, Clarence M. Jackson; for Vice-President, Harold D. Senior; for Secretary-Treasurer, Lewis H. Weed; for members of Executive Committee, terms expiring 1925, Davenport Hooker and Benjamin F. Kingsbury.

On motion, the Secretary was instructed to cast a ballot for the election of the above named.

The Secretary then announced that the election of Lewis H. Weed as Secretary-Treasurer caused a vacancy on the Executive Committee for a two-year term. The President then called for a nomination to fill this vacancy.

Professor Irving Hardesty was nominated and elected to serve on the Executive Committee until 1923.

The Secretary presented the following names recommended by the Executive Committee for election to membership in the American Association of Anatomists:

- ADAMS, L. A., Ph.D., Instructor in Comparative Anatomy, *University of Illinois, Urbana, Ill.*
- BAST, T. H., B.A., Ph.D., Assistant Professor of Anatomy, *University of Wisconsin, Madison, Wis.*
- CAMERON, ANGUS L., M.D., Ph.D., Assistant Professor of Surgery, *Medical School, University of Minnesota, Minneapolis, Minn.*
- CALKINS, LEROY A., M.D., Ph.D., Assistant Professor of Obstetrics, *Medical School, University of Minnesota, Minneapolis, Minn.*
- DUNN, H. L., M.A., *Department of Anatomy, University of Minnesota, Minneapolis, Minn.*
- GATENBY, J. BRONTE, Ph.D., Professor of Zoology, *Trinity College, Dublin, Ireland.*
- GEIST, FREDERICK D., M.D., Instructor in Anatomy, *University of Wisconsin, Madison, Wis.*
- GREEN, R. B., M.B., F.R.C.S., Demonstrator of Anatomy, *University College, Gower St., London, W. C. 1, England.*
- HANSON, FRANK BLAIR, Ph.D., Associate Professor of Zoology, *Washington University, St. Louis, Mo.*
- HARWELL, CHAS. S., A.B., M.D., Assistant Professor of Anatomy, *Emory University, 435 Euclid Ave., Atlanta, Ga.*
- HUNTER, JNO., M.B., Ch.M., Anatomy Department, *Sydney University, Sydney, Australia.*
- HUBER, ERNST, Ph.D., Associate in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- HOY, WM. E., JR., Ph.D., Professor of Biology, *Presbyterian College of S. Carolina, Clinton, S. C.*
- HUMPHREY, RUFUS R., A.M., Instructor in Histology and Embryology, *Cornell University, Ithaca, N. Y.*
- LEE, FERDINAND CHRISTIAN, A.B., M.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- MILLER, SHIRLEY P., A.B., A.M., Instructor in Anatomy, *Anatomical Institute, University of Minnesota, Minneapolis, Minn.*
- MULL, PHILIP L., B.S., M.D., Instructor in Anatomy, *University of Louisville, Medical Department, Louisville, Ky.*
- NITTONO, K., M.D., Professor of Anatomy, *Chiba Medical College, Chiba, Japan.*
- NICHOLSON, F. M., Ph.D., Assistant in Pathology and Bacteriology, *Rockefeller Institute for Medical Research, 66th Street and Avenue A, New York.*
- ROMER, ALFRED S., B.A., Ph.D., Instructor in Anatomy, *University and Bellevue Hospital, Medical College, 338 East 26th St., New York City.*
- RIENHOFF, WILLIAM F., JR., A.B., M.D., Instructor in Surgery, *Johns Hopkins Hospital, Baltimore, Md.*
- SMITH, BERTRAM GARNER, A.B., Ph.D., Associate Professor of Anatomy, *University and Bellevue Hospital Medical College, 338 East 26th Street, New York City.*

- SMITH, CHRISTIANNA, A.M., Instructor in Zoology, *Mt. Holyoke College, South Hadley, Mass.*
- STARK, MARY, Ph.D., Professor of Histology and Embryology, *New York Homeopathic College, New York City.*
- STEVENSON, PAUL H., M.D., Assistant in Anatomy, *Peking Union Medical College, Peking, China.*
- SISSON, SEPTIMUS, B.S., V.S., Professor of Veterinary Anatomy, *Ohio State University, Columbus, Ohio.*
- SYKES, GEO. F., Ph.B., M.A., Professor of Zoology and Physiology, *Oregon State Agricultural College, Corvallis, Oregon.*
- UHLENHUTH, EDUARD, Ph.D., Associate in Pathology, *Rockefeller Institute for Medical Research, 66th Street and Avenue A, New York City.*
- WHITESIDE, BEATRICE, Ph.D., Instructor in Anatomy, *Washington University Medical School, St. Louis, Mo.*
- WOODMAN, A. S., M.D., Assistant Professor of Histology and Embryology, *Boston University School of Medicine, Boston, Mass.*

On motion, the Secretary was instructed to cast a ballot for all the candidates proposed by the Executive Committee. Carried.

The Secretary then announced the following names as dropped from the list of members on account of non-payment of dues for the past two years:

- DR. E. A. BAUMGARTNER, Halstead, Kansas.
- DR. W. B. CHAPMAN, Washington University, St. Louis, Mo.
- DR. ELBERT CLARK, University of Chicago, Chicago, Ill.
- DR. FABIO FRASSETTO, University of Bologna, Italy.
- CAPT. C. F. SILVESTER, Army Medical Museum, Washington, D. C.

It was announced that the Executive Committee had voted to hold the next annual meeting during Easter week of 1923 in the central part of the country, or Mississippi Valley, the place to be determined later.

The Executive Committee nominated four persons, two of whom were to be elected by the Association for membership on the Journal Committee as organized at the previous meeting. As a result of the election, Professor F. T. Lewis was chosen to serve for five years and Professor H. M. Evans to serve for four years as members of this committee.

It was moved by Professor H. H. Donaldson and seconded, That the Association express to the retiring Secretary-Treasurer its sincere appreciation of his efforts for, and loyalty to, the

Association during his years of service. The motion was approved by a rising vote.

On motion, the business session then adjourned.

FRIDAY, DECEMBER 30. A BUSINESS SESSION followed the morning Scientific Session.

A discussion regarding the publication in the Proceedings of the Meeting of abstracts by persons not attending the sessions was introduced. It was finally resolved that in the future those members not intending to be present at the meeting should send in their communications as to be read by title only. The abstracts of such communications are to be published in connection with the proceedings of the meeting.

Professor G. L. Streeter introduced the following resolution:

RESOLVED: That the Association express to the authorities of Yale University its sincere appreciation of the exceptional facilities and accommodations that have been provided for this meeting, and which have in so large part contributed to its success, and also for the cordial hospitality of Professor Harrison, Professor Ferris, and the members of the local committee that has been so generously extended to all those in attendance.

Unanimously voted.

On motion, the Session adjourned.

CHARLES R. STOCKARD.

Secretary of the Thirty-eighth Session of the  
American Association of Anatomists





## ABSTRACTS

1. *A comparison of the cerebellar tracts in three teleosts.* WILLIAM H. F. ADDISON, University of Pennsylvania.

The cerebellar fiber-tracts of three fishes of different habits have been studied, in order to compare the relative sizes attained by each tract in the three species. The three fishes were *Gadus morrhua* (cod), *Arius* (a small Siluroid), and *Pleuronectes limanda* (flounder). Serial sections were studied in the Netherlands Central Brain Institute of Amsterdam, by courtesy of Dr. Ariens Kappers, director. The afferent cerebellar tracts in the teleostean brain are: mesencephalo-cerebellaris anterior, mesencephalo-cerebellaris posterior, lobo-cerebellaris, spino- et olivo-cerebellaris, vestibulo-cerebellaris, and laterali-cerebellaris. The efferent cerebellar tract is the cerebello-motorius, of which the most anterior part is the brachium conjunctivum anterius. Two examples illustrating the nature of the study may be given. The mesencephalo-cerebellaris anterior is largest in cod, intermediate in flounder, smallest in *Arius*. This tract arises in part from the tectum opticum, transmits impulses from the visual centers, and is largest in the form having the largest visual apparatus. The mesencephalo-cerebellaris posterior is largest in *Arius* and less well-developed in the other two. This tract arises near the end-station of the lateral lemniscus, probably transmits impulses from the latter, and is largest in the form having the best-developed lateral-line organs. In this way, one may correlate in size the various afferent tracts with the receptors from which they transmit impulses to the cerebellum, and may gain evidence as to the relative part played by the different tracts in the formation of the cerebellum.

2. *Growth of the medullary tube grafted into the allantois.* (Lantern.) ANNA AGASSIZ and VERA DANCHAKOFF, Columbia University.

The development of the spinal cord manifests a high degree of independence from its continuity with the cranial part of the central nervous system. A number of grafts of blastoderms were made at different stages, beginning with 5 somites. The part of the blastoderm which contained the four or five, sometimes a greater number of cranial somites was severed and the caudal part, containing 1 to 10 somites, grafted into the allantois. That part of the neural tube which had been closed at the time of grafting invariably grows and differentiates in a manner similar to that in which it does normally. It is more difficult at present to conclude whether the ectoderm caudally to the somites will as readily differentiate. Not only does the neural tube differentiate white and grey matter in the graft, but, as demonstrations will show, the grey matter differentiates into ventral and dorsal horns, and even various cell groups in these can be recognized. Interesting data in relation to the migration of groups of nerve cells are obtained, inasmuch as groups of nerve cells are found scattered in the graft through regions of more or less undifferentiated tissue. Nerve trunks grow without reaching the organs they would normally have supplied, as these organs

often fail to develop. Neither continuity with the cephalic part for the differentiation of the spinal cord, nor presence of developing organs for migration of nervous ganglia and growth of nerve trunks seems to be required for growth and development of the nervous tissue in the embryo.

3. *The morphogenesis of the hypophysis in the tailed amphibia.* WAYNE J. ATWELL, Medical Department, University of Buffalo.

The hypophysis has been studied in *Ambystoma*, *Spelerpes*, *Necturus* and *Amphiuma*. The most striking difference between these forms and the *Anura* previously studied is in regard to the adult condition of the *pars tuberalis*. As in the *Anura* this part has its origin from a pair of processes which grow forward from the remainder of the gland. These processes do not become detached to form separate epithelial plaques as they do in the frog and the toad at the time of metamorphosis, but maintain their connection with the anterior lobe throughout life. The anterior lobe proper and the *pars intermedia* develop as in the *Anura* except that the adult position of the *pars intermedia* is dorsal to the anterior lobe rather than caudal to it. The neural lobe is considerably sacculated in *Necturus* and *Ambystoma*. Judging from this criterion alone, these two forms are primitive and are rather closely related to certain of the fishes.

4. *Autoplastic transplants of the epithelial hypophysis in larvae of Rana pipiens.* WAYNE J. ATWELL, Medical Department, University of Buffalo.

The epithelial hypophysis was transplanted from the head to the tail in very young tadpoles of *Rana pipiens*. The operated animals failed to develop the typical silvery appearance of the hypophysectomized tadpole. They were, however, lighter in color than normal larvae. Examination of the living tadpole under the binocular microscope shows that the melanophores are not so contracted nor the xantholeucophores so widely expanded as in the typical silvery larva. The appearance is that the hypophysis, or at least the *pars intermedia*—which is responsible for the color change—has been only partly compensated for by the transplant. The condition is evidently that of a chronic hypophyseal insufficiency. Serial sections show that the hypophysis was completely removed from the head. The region of the transplant in the tail is readily observable macroscopically because of the expanded condition of the melanophores in the immediate vicinity. This probably indicates a local effect of the transplanted gland. Although the transplant was apparently functioning more than four months after the operation none of the tadpoles underwent metamorphosis. Whether this is due to the atrophy of the anterior lobe tissue in the transplants can not be stated because the degree of histological differentiation is not sufficient to permit identification of the separate parts. It is possible, however, to identify two varieties of cells in the transplant, both apparently parenchymal.

5. *The increased absorption of x-ray energy by vitally stained white rats.* W. M. BALDWIN, Union University (Albany) Medical College.

Paramoecia which are vitally stained indicate an increased susceptibility to the action of x-ray energy. This investigation represents an effort to establish a general principle covering the absorption by living tissues of x-ray energy increased through chemical means. The source of this energy was a large Cool-

idge water-cooled tube carrying a load of 50 Mam. at 50 K.V. The rats were placed 17.5 cm. from the center of the target and each was rayed for two consecutive minutes. A dosage of 100 Mam. minutes was sufficient to induce death at an average of from 100 to 120 hours after the exposure. When such vital stains as trypan blue, trypan red, neutral red, isamine blue, etc., were used in an amount not sufficient to affect the vitality of the rats, a dosage of 100 Mam. minutes induced death within from forty-eight to fifty-six hours. This increase in the absorption of x-ray energy conforms to the results obtained by Hertel studying bacteria sensitized to radiant energy of the length of ultraviolet light rays.

6. *The sitting height. Based on the measurements of more than 225,000 individuals.*

R. BENNETT BEAN, University of Virginia.

*Age.* Girls are precocious and boys retarded in the growth of the sitting height. The sitting height grows more rapidly at and after puberty than before. It remains stationary from 25 to 60 years and decreases thereafter. The hypophylomorph is retarded and the hyperphylomorph precocious in the growth of the sitting height, and the mesophylomorph is intermediate. The Asiatic has the sitting height accelerated early and retarded late in growth, the growth of the sitting height in the African is late and short, and the European is intermediate. The Asiatic has a long torso and short legs, the African a short torso and long legs, and the European is intermediate, in the adult.

*Race and type.* We may group the races by type:

Extreme hypophylomorph: Aino, Lapp, Pigmy, Eskimo, Siberian, Aymara.

Hypophylomorph: Southeast Asia. Some negroes and Negritos.

Mesophylomorph: American Indian. Central Asia. Some negroes.

Hyperphylomorph: European, Indo-European, Nordic and derivatives.

Extreme hyperphylomorph: Mediterranean race and related stocks.

Each race has hypo stocks, and some have meso and hyper. The Lapps, Finns, and Ainos are the hypos for Europe, the Alpine and sub-Nordics are the mesos, and the Nordic and Mediterranean are the hypers. The Pigmies are the hypos for Africa, the Central Africans are the mesos and the long-legged Negritos are the hypers. The Siberians and southeast Asiatics are the hypos for Asia, the Chinese are the mesos, and the Thibetans and Japanese are the hypers.

Race movements have altered the type.

7. *Contraction rate of the uterine musculature of the rat with reference to the oestrous cycle.* EDWARD W. BLAIR (introduced by Herbert M. Evans), Department of Anatomy, University of California.

The spontaneous rhythmical contractions of the excised rat uterus (one horn), suspended in warm (38°C.) oxygenated Locke's solution, were recorded by means of a heart lever writing on a long-roll kymograph. The rhythm is found to be slowest at the time of oestrus, increasing gradually to a maximum late in the resting stage, and decreasing rather suddenly at the pro-oestrus. The contraction and relaxation time are little affected, the difference being in the interval between contractions. The rhythm of the resting uterus is often broken by the appearance of superimposed contractions before relaxation is complete. These were ignored in estimating the rate. Barely perceptible minimal contractions are often interpolated between the ordinary maximal contractions of the uterus,

at and near oestrus. These also are ignored in the data tabulated. Age and history of previous pregnancies also influence the rhythm, but do not affect the influence of the oestrous cycle.

SMEAR	NUMBER OF ANIMALS OBSERVED	MEAN INTERVAL BETWEEN CONTRACTIONS	RATE PER HOUR
		<i>minutes</i>	
Small epithelial cells only (Pro-oestrus).....	2	2.34	25.6
Small epithelial and cornified cells (Oestrus).....	5	2.5	24.0
Cornified cells only (Post-oestrus).....	4	1.42	42.3
Leucocytes and epithelial cells (Early Di-oestrous interval).....	5	1.06	56.6
Leucocytes and epithelial cells (Late Di-oestrous interval).....	2	0.77	77.9

8. *The pancreatic ducts of the white rat.* J. L. BREMER, Harvard Medical School.

The pancreas of the white rat surrounds the hepatic duct throughout almost the entire extent of the latter from liver to duodenum, and is connected with it by numerous ducts. These all represent ventral pancreas, as the duct of the dorsal pancreas degenerates early. The original relations of liver diverticulum, single ventral, and dorsal pancreas are similar to those in other mammals. Besides these true pancreatic ducts, of varying size, many smaller blind gland-like pockets open into the hepatic duct.

9. *The control of head formation in Planaria dorotocephala by means of anesthetics.*

J. W. BUCHANAN (introduced by R. G. Harrison), Yale University.

By mass experiments it is shown that head frequency in pieces of *Planaria* can be controlled by subjecting the pieces for short periods after section to appropriate concentrations of chloretone, chloroform, chloral hydrate, ether, and ethyl alcohol; that in such concentration of these agents the increase of oxygen consumption following section does not occur.

The evidence shows that the factors controlling head formation are non-specific and supports Child's conclusions regarding the nature of these factors, viz., that head formation is determined by the relative activities of two antagonistic factors: 1) the tendency of the cells near the anterior cut surface of the piece to de-differentiate and to develop into the head of a new individual; 2) the tendency of the whole of the piece, exclusive of the cells concerned in the development of the new head, to maintain the differentiation of the old individual, exerting a certain degree of control over the cells near the anterior cut surface and tending to prevent the development of a new head.

The facts indicate that anesthetics alter head frequency, 1) by direct inhibition of the processes of development of the cells concerned in the formation of the new head, causing decreases in head frequency; 2) by inhibition of the increase of metabolic activity of the whole of the piece following section; in pieces from

certain regions this effect overbalances the direct effect on the developmental processes of the cells concerned in forming the new head and in such pieces head frequency is increased.

10. *The fate of the neuropore in Amblystoma embryos.* H. S. BURR, The School of Medicine, Yale University.

A study during the past few years of the early stages in morphogenesis of the cerebral hemispheres in *Amblystoma* has confirmed the findings of Kingsbury ('20) concerning the rostral relations of the primary columns of His, and indicated the possibility of an experimental solution to the question of the fate of the neuropore. Small wounds made in the ventral lip of the neuropore and stained with Nile blue sulphate could be followed for many days during subsequent development. By comparing careful camera lucida drawings of such operated embryos, with median sagittal sections of the same it was possible to locate with precision the ultimate position occupied by the lips of the neuropore. The ventral lip of the neuropore is incorporated into the neural tube as the terminal ridge (Johnson '09). The lateral lips of the neuropore fuse to form the lamina terminalis. It is evident, therefore, that the lamina terminalis is the terminal portion of the roof plate of His which by this fact ends at the terminal ridge. Furthermore, the alar plates forming as they do the lateral boundaries of the roof plate sweep, in the terminal ridge, across the mid line thus bringing the alar plate of one side into direct continuity with that of the opposite side. This means that the mid ventral region of the rostral end of the neural tube is, in *Amblystoma*, occupied by the alar plate as far caudally as the preoptic recess and not by the floor plate.

From this latter point, the terminus of the sulcus limitans, the mid ventral line of the neural tube as far caudad as the fovea isthmi shows in *Amblystoma* none of the characteristics of floor plate material. Rather as Kingsbury ('20) has shown in other forms, this region is occupied in the main by nervous tissue which is continuous, in the early stages with the basal plate of the neural tube.

We find in *Amblystoma*, then, that the rostral relations of the primary columns of His are as follows: The roof plate terminates at the terminal ridge and the floor plate at the fovea isthmi. The alar plates are continuous from side to side in the terminal ridge, and the basal plates in the region from the fovea isthmi to the preoptic recess show a similar continuity.

11. *The development of the nasolacrimal apparatus in Amblystoma.* H. S. BURR and E. S. A. ROBINSON, The School of Medicine, Yale University.

An experimental study of the development of the nasolacrimal apparatus in *Amblystoma* has revealed the following facts. The entire canal, including the superior and inferior lacrymal ducts, is laid down as a thickening of the inner layer of the ectoderm along the bottom of the naso-optic furrow. By proliferation and delamination the thickening develops into a solid cord of cells which begins to separate from the ectoderm at the nasal end. This separation is never entirely completed as the ocular end retains its connection through subsequent growth. Eventually the nasal end of the cord sinks through the mesenchyme of the head to become attached to the nasal epithelium. Lumen formation starts in the solid cord usually at the ocular end by tangential mitoses of the peripheral cells and migration of the central cells into the interstices between the former.

Operations involving the removal of the eye in one series, of the nose in a second, yielded the following results. In each type of operation the removal of the sense organ involved also the removal of the primordium of the lacrymal apparatus since the eye, and the nose at this stage of operation are so closely related that the ectodermal site of the apparatus is usually removed when either is extirpated. The absence of either the ocular or the nasal placode associated with removal of the naso-optic furrow results in the absence of these passages, hence neither sense organ is a determining factor in regeneration of the nasolacrymal apparatus. Incomplete removal of the optic-furrow results in partial regeneration of the passages irrespective of the presence or absence of the sense organs.

*12. The topography of the abdominal viscera in the late embryonic and the fetal period of man as determined by graphic reconstruction.* (Lantern.) ANGUS L. CAMERON (introduced by R. E. Scammon), Department of Anatomy and Department of Surgery, University of Minnesota.

The topography of the abdominal viscera of fifty selected specimens of the latter part of the embryonic and of the fetal period was determined by means of graphic reconstructions with the orthoscopic apparatus and by field graphs of the vertebral levels of the various portions of the abdominal organs.

The transition from the embryonic to the fetal period is characterized by a number of marked changes in abdominal topography. These are completed, in the main, by the close of the third month, and during the rest of the fetal period the relations of the viscera to skeleton and to each other remain remarkably constant. The chief changes in abdominal topography in the fetal period are those of the caecum, splenic flexure and sigmoid flexure. The axis of the spleen is also somewhat modified and the head of the pancreas is depressed.

These results fall in line with the findings of other workers in this laboratory that the growth of the external proportions of the abdomen follows a constant course in the fetal period. The particular changes enumerated above seem to be principally mechanical ones dependent on the distension of the colon with meconium in the latter months of prenatal life.

*13. Studies in the dynamics of histogenesis. VII. The experimental transformation of the smooth bladder muscle of the dog, histologically into cross-striated muscle and physiologically into an organ manifesting rhythmicity.* E. J. CAREY, Marquette School of Medicine.

1. The essential difference between the pale smooth muscle of the bladder and the red involuntary striated muscle of the heart is finally dependent upon the differential intensity of hydrodynamic tensional stimuli (work) to which the vesicular and cardiac mesenchymal cells are subjected, respectively. If the growing vesicular smooth muscle is subjected to a stimulus comparable to that found in the heart, will cross-striated muscle be differentiated? The answer is yes.

2. The evidence presented proves definitely that the pale bladder musculature may be transformed into the red, cross-striated type by increasing the tensional stimulus to a degree comparable with that which the cardiac mesenchyme experiences normally.

3. Muscle tissue is not a self-differentiated product, but is a biomechanical resultant of an optimum tension. The variable intensity of the optimum tension determines the muscular type. The growing cells receive and respond to the mechanical tensional stimulus. The stimulus, however, is a function of position.

4. The evidence herein presented proves that the structure of striated muscle is determined by the function it performs and the work it does and that cross-striated muscle is not formed in anticipation to a future function. The conclusion is warranted that function in this case determines structure, and not the reverse.

14. *Transformations of the branchial arteries in man.* E. D. CONGDON, Stanford University Medical School.

Owing to the subtlety of the changes in the walls of the branchial arteries, there are very few portions of the carotids or of the definitive aortic arch that can with certainty be allotted to specific parts of the branchial system. This is illustrated by the changes taking place after the disappearance of the first and second branchial arteries, when the third, fourth, and sixth radiate upward from a pouch and the primitive external carotid comes off at the junction of the third artery and the pouch. The region of union of the third and fourth branchial arteries and the external carotid is seen to migrate upward. This is apparently due in part to the remodeling of the pouch wall into a tube. Perhaps there is also a blending of the lower ends of the two branchial vessels. By a similar process the point of union of the primitive external carotid with the third branchial artery is in turn carried away from the fourth by the formation of an intermediate trunk, the common carotid.

15. *The reticular material as an indicator of physiologic reversal in secretory polarity in the thyroid cells of the guinea-pig.* E. V. COWDRY, Rockefeller Institute for Medical Research, New York.

In the thyroid glands of normal guinea-pigs, the reticular material (i.e., Golgi apparatus, canalicular apparatus, etc.) is not invariably found between the nuclei and the follicular lumen, as is generally supposed; but in some cases undergoes an active migration to the opposite pole of the cell, which, together with other evidence at hand, indicates the existence of a reversal in physiologic polarity whereby the secretion is discharged directly into the circulation, instead of being first stored within the follicles.

16. *Studies on the spleen.* R. S. CUNNINGHAM. Department of Anatomy, Johns Hopkins Medical School.

a. *The rôle of the spleen in experimental hydremia.* In two series of experiments on cats hydremic plethora was produced by continued intravenous injection of physiological saline. In normal animals about 40 cc. of fluid collected in the peritoneal cavity before there was any in the pleural or pericardial cavities. In animals whose spleens had been previously removed fluid appeared simultaneously in all three cavities. It was also found that a considerably larger amount of fluid must be injected in splenectomised animals in order to obtain an ascites equal to that produced in normal animals. In contrast to the other abdominal organs the spleen apparently never became edematous even in those



experiments in which large amounts of fluid were injected. The mesothelial cells of the spleen, from animals in which an ascites was developing, were found to contain vacuoles of various sizes. In certain experiments in which iron ammonium citrate and sodium ferrocyanide were injected intravenously granules of Prussian blue were found in these vacuoles. *b. Reaction of the splenic mesothelium to vital dyes.* In animals that had been vitally stained by intravenous injections the splenic mesothelial cells contained the dye in fine perinuclear granules. After irritation the cells became cuboidal and finally columnar, and the granules of vital dye were collected principally in the infranuclear zone of the cell. This location of the vital dye inclusions was very similar to, but considerably less in amount than, that found in the germinal epithelium of the ovary.

17. *Grafts in the allantois of embryonic anlagen of the chick.* (Lantern.) VERA DANCHAKOFF, Columbia University.

Young blastoderms in toto, parts of blastoderms at various stages and chick anlagen of different organs, were grafted into the allantois. The earlier a whole blastoderm is transferred to the allantois, the less growth and differentiation is obtained. It seems as if energetic growth of embryonic cell groups can be effected in the allantois only after a definite organization of the embryonic cell material has been completed. Such organization fails to take place in the allantois, if the blastoderm is transferred into it at a stage of early primitive streak, and the growth resulting from such a graft contains only islands of undifferentiated tissue. If, however, the blastoderm is allowed to develop to an advanced primitive streak stage showing a slight indication of the head process and is then grafted the different cell groups grow well and reach a high degree of differentiation.

Among such cell groups it is especially those which develop into kidney, nervous tissue, eye and notochord, which seem to manifest the least disturbing effect from the transfer of the blastoderm into the allantois. The segment stalks develop tubules and Malpighian glomerules, the neural plate differentiates gray and white matter, sends out nerve trunks, and groups of nerve cells migrate far away into various parts of the graft, the visual organ develops pigment and lens; the notochord attains a considerable size and becomes surrounded by cartilage; a cartilaginous envelope is formed around the spinal cord and in this different parts of normal vertebra are recognized.

Separated parts of the blastoderm also grow on the allantois. A curious result was obtained in relation to the pronephros. Blastoderms with 10 and more somite embryos were cut into two parts, between the 8th and 9th somite, and the cranial part with the anterior 7-8 somites grafted. In this case the pronephros seems to attain a more extensive development than that reached by the pronephric anlage under normal conditions.

18. *The question of digital homology.* C. H. DANFORTH, Washington University School of Medicine.

The character of a digit appears to be regulated by at least two different factors. One is the tendency of limb-buds to produce terminal lobulations, the other is something in the general nature of gradients between the pre- and post-axial borders. The 'gradients' of the upper and lower limbs are apparently equivalent, but the lobulations do not seem to be so. When the hand and foot are

compared it becomes apparent that, if the digits of the two limbs are considered as equivalent units, many comparable features are nearer the preaxial side of the foot than of the hand. Thus, in the hand the longest digit is the third; in the foot, usually the second. Hair centers for the mid-digital segments are on the fourth finger and the third toe. The Mm. interossei are oriented with reference to the third finger and the second toe. Other muscles and bones show similar tendencies, although less clearly. Simple hereditary syndactyly involves the third and fourth fingers, the second and third toes. Accessory tendons from the preaxial side reach the second finger oftener than they reach the second toe. Tendons to the fifth toe are suppressed more frequently than those to the fifth finger. These facts, with the strongly corroborative evidence from polydactyly, are interpreted to mean that the digits of the hand are not serially homologous with those of the foot, the great toe being equivalent to rather more than the thumb, the little toe to somewhat less than the little finger.

19. *Some significant features of the anatomy of a symelian monster (Sympus monopus).* ALDEN B. DAWSON, Loyola University School of Medicine, Chicago.

Ballantyne ('04) has shown that fetuses which have only one umbilical artery almost constantly present the malformation known as sympodia. He believed that the single artery of the cord was vitelline in origin and accordingly concluded that the arterial supply of the placenta must be non-allantoic. In the case under discussion, however, the single umbilical artery appeared to be formed by the fusion of two umbilical arteries. The single trunk took its origin from the aorta a short distance below the superior mesenteric, the inferior mesenteric appearing as a branch of the umbilical. The relatively high origin of the umbilical artery can be explained as a retention of the early embryonic position. Caudally the umbilical trunk gave off a single iliac trunk which divided to supply the fused lower limbs.

There were no genito-urinary or anal apertures. Both testes were present, but the right was undescended. Their vasa deferentia opened into the blind end of the digestive tract. The right kidney was absent. The left was rudimentary, and its ureter opened into the left vas. Both suprarenal glands were present and their arterial supply was of the embryonic type.

The caudal end of the vertebral column was suppressed and the spinal cord extended the entire length of the canal, i.e., to the level of the fourth sacral vertebra. The segmentation of the thoracic region was disturbed. Extensive fusion occurred in the pelvic girdle. There was a single median femur, a single median fibula and two lateral tibiae.

The anomalies which are constant for this group of monsters will be described in detail, and their developmental significance discussed.

20. *The standardization by empirical formulae of the data on surface area in man and animals.* H. L. DUNN (introduced by R. E. Scammon), Department of Anatomy, University of Minnesota.

This study is limited to a consideration of the recorded data on the surface area of man and animals. The material obtained was analyzed graphically and its expression developed by the use of empirical formulae.

The animals in which the relation of body weight to surface area has been determined are cattle, guinea-pigs, rats, rabbits, frogs and lizards. However, the data on these forms are not sufficient to permit a comparison with human material. In man the relationship of surface area to body weight, body length and time in years has been studied by four classes of methods: *a*) the paper and mould methods which are the most accurate since they closely approximate the surface; *b*) the geometric method, which is only of approximate accuracy; *c*) the linear method of DuBois based on empirical measurements; *d*) the photographic method of Benedict which involves the application of DuBois' linear formula to photographic silhouettes.

Conclusions: 1) The results of the paper and mould methods must be regarded as the most reliable. 2) The curve of the central tendency of all data on the relation of surface area to body weight in man may be expressed by the general empirical formula:  $Y = 0.02 X^2 + 3.9 X + 10$  in which  $Y$  is square decimeters,  $X$  kilograms, and 10 is an empirically determined constant.

*21. The azurophile granulation of the lymphoid cells of the blood during digestion and fasting and in relation to the tributaries of the venous system.* V. E. EMMEL, Department of Anatomy, University of California.

A quantitative study of the azurophile granulation in the lymphoid cells of the circulating blood of the rat was made with reference to digestion and fasting, together with a comparative analysis of the blood in the tributaries of the venous system with the following results:

First. That in any given animal under constant physiological and dietary conditions the percentage of lymphoid cells showing azurophilic granulation, as tested for the peripheral circulation from day to day, is relatively constant. Second. In animals fasting for 24 to 90 hours there is a decrease in the percentage of azurophile granulation followed by a pronounced increase during digestion. Third. In animals under normal physiological conditions the inferior cava, superior to the hepatic veins, constantly gave a higher percentage of azurophile granulation as compared with the superior cava. Fourth. Upon analysis of the tributaries of the inferior cava, the portal vein was found constantly higher in the percentage of azurophile granulation than that of the abdominal part of the inferior cava; the latter being practically equal to that of the peripheral circulation. Fifth. On the other hand, in fasting animals this difference, with respect to the portal vein, largely disappeared, so that the azurophile granulation for the portal vein was now essentially the same as that of both the peripheral circulation and the inferior cava inferior to the hepatic veins. Sixth. The above differences based upon the percentage of the total number of lymphoid cells containing azurophile granulation is further confirmed and emphasized by corresponding differences in the numerical content of azurophile granules within these cells.

A further analysis of the tributaries of the portal system, together with the effect of different diets, is under investigation.

22. *The percentage of polychromasia in the blood of the rat as correlated with gestation, lactation, and the oestrous cycle.* V. E. EMMEL and J. E. WALKER, Department of Anatomy, University of California.

In a number of mammals a certain percentage of the erythrocytes in the circulating blood, normally show a polychromatophilic reaction to basic dyes. As this polychromasia is especially pronounced in the rat, it has been utilized as an index in determining to what extent there may be an interaction between the activities of the reproductive system and the cellular elements of the blood in this animal. The present stage of this investigation shows the following results:

A. Successive blood tests for any given normal male or non-pregnant female gave a relatively constant percentage of polychromasia. B. In female rats, during gestation, there was a definite rise in the percentage of polychromasia. This increase was further accentuated during lactation. In both instances, this increase appeared most pronounced in the earlier phases of these stages. C. Fluctuations in the total erythrocyte count seemed, in general, to correspond with these changes in the percentage of polychromasia. D. A control series of non-pregnant animals failed to show changes corresponding to the pregnant and lactating animals. E. In tests made during the oestrous cycle the percentage of polychromasia was always slightly higher at the pro-oestrous stage and correspondingly lower at the following di-oestrous stage. F. In a series of observations on postnatal changes in the blood of three males the percentage of polychromasia gradually declined from an average of 21.3% at birth to 5.1% on the 56th day. This is suggestive of a correlation between the postnatal reduction in polychromasia with the attainment of sexual maturity—a conclusion which remains to be confirmed by further investigation.

23. *On an invariable and characteristic disturbance of reproductive function in animals reared on a diet poor in fat soluble vitamine A.* HERBERT M. EVANS and KATHERINE SCOTT BISHOP, Department of Anatomy, University of California.

Studies herein reported confirm the impression that rats may be successfully reared on diets poor in vitamine A if the diet is not too deficient in this essential. They may for months grow normally and not suffer from the so-called xerophthalmia. It must be admitted that we have not had previously a method for the detection of deficiencies in this vitamine which, nevertheless, permit good growth and apparent health. Studies on the oestrous cycles of such rats show them to suffer from an invariable and continuous abnormality or disfunction of the ovaries.

It has previously been shown in this laboratory that ovulation in the rat can be detected in the living animal by a series of histological changes in the vaginal smear, changes which are correlated with the growth, maturation and rupture of the graafian follicles at periodic intervals. When for any reason the follicles are unable to completely mature (as in animals treated with hypophyseal substance) the vaginal oestrous changes are absent. If follicles develop but are unable to rupture, the oestrous changes may be remarkably prolonged, and the dioestrous pause, in fact, obliterated. As Evans and Long have shown, this occurs as a rare anomaly in large colonies of animals. But this prolongation of oestrous vaginal changes and failure of ovulation occurs in 100 per cent of animals

reared on diets which are low in vitamine A but which nevertheless permit normal growth. We have used typical diets employed by E. V. McCollum and by T. B. Osborn and L. B. Mendel, in which the chief fat content was furnished by lard. On the administration of small quantities of dried, powdered leaves of young succulent alfalfa or of small quantities of butter fat, this characteristic abnormality in oestrus and ovulation was cured.

24. *Oestrus and ovulation in the rat, from a large group of animals on a standard diet.* HERBERT M. EVANS and KATHERINE SCOTT BISHOP, Department of Anatomy, University of California.

It is important to know what might be expected to be the normal oestrous behavior of animals reared from the time of weaning on a satisfactory standard diet. For this purpose we have employed a standard ration used by E. V. McCollum, consisting of whole wheat, whole milk powder, casein, salts and 5 per cent butter fat. Many animals have been maintained on this ration as littermate controls of animals on other nutritive regimens, so that there is available for these data the performance of over five hundred individuals. The growth exceeds considerably that reported by other investigators as normal. Daily microscopic observations were made on the vaginal smears. The average time of occurrence of the first oestrous cycle in the group was on the forty-seventh day of life.

*Occurrence of first oestrus in 570 individuals*

AGE IN DAYS	NUMBER OF INSTANCES	PER CENT
32-36	18	3.1
37-55	459	80.5
56-66	73	12.8
67-94	20	3.5

*Length of oestrus cycle in 10,000 instances*  
(First four cycles in each history are omitted)

LENGTH OF CYCLES IN DAYS	NUMBER OF INSTANCES
3	72
4	3,494
5	3,943
6	1,439
7 and over	1,052
	10,000

General average: 5.4 days.

It will be noted that the sexual maturity of these animals which were maintained on a superior nutrition is precocious when compared with the standards known to the literature. In the majority of cases of exceptionally early maturity

there is a superior growth rate. It is also true that the slowest growers usually mature later. The latter statement is firmly established by our work (not presented here) on quantitative and qualitative under-nutrition which invariably delays sexual maturity.

An examination of the normal curve of growth with reference to the time of establishment of ovulation shows that the rate of growth does not change with this event.

**25. Characteristic effects upon growth, oestrus, and ovulation induced by the intraperitoneal administration of fresh anterior hypophyseal substance.** HERBERT M. EVANS and JOSEPH A. LONG, University of California.

At the last session we reported acceleration of growth in rats treated daily intraperitoneally with the finely ground, fresh anterior lobe of the hypophysis of beef. There is lack of effects from oral administration of the same substance. Treated animals are invariably heavier than their litter mate sisters. The greatest disparity was attained on the 333rd day of life, when an animal receiving anterior hypophyseal substance weighed 596 grams and its healthy litter mate control weighed 248 grams. It would not appear to be incorrect to characterize these changes as producing constantly a mild degree of gigantism. Increase in weight results to a great extent from a storage of fat, but is not solely due to this, the skeleton being invariably somewhat larger and heavier, and, as would be expected, the heart, lung, alimentary canal and kidney are heavier. The weight of the hypophysis, thyroid and thymus are not appreciably affected.

Oestrus, as detected by typical changes in the vaginal smear, may never occur in these animals or may be exhibited only at long intervals. In all instances the ovaries instead of being underdeveloped weighed twice as much as the control and exhibited great numbers of substantial corpora lutea. The uterus, on the contrary, weighed absolutely about half as much as it did in the normal controls. Histological examination of the gonads showed abundant lutein tissue and demonstrated the formation of this tissue about the egg in unruptured, normal follicles and in atretic follicles. Ripe, normal Graafian follicles were invariably absent. A powerful, specific stimulus to lutein cell transformation has thus been effected by this hormone.

**26. A strain of epithelial cells in pure culture.** (Lantern.) ALBERT FISCHER, (introduced by E. V. Cowdry), Rockefeller Institute for Medical Research.

By cultivating in vitro parts of the chick embryo lens containing cells from the iris, a pure outgrowth of epithelial cells was successfully obtained. The strain was derived from a little rim of iris which spontaneously adhered to the lens when removed. It has been cultivated for more than two months, and is still proliferating in a way which is characteristic of epithelial growth in vitro. No connective-tissue cells have been observed. The culture medium is the same as has been used for cultivating fibroblasts, but by allowing the cells to multiply on the surface, instead of in the plasma clot, a more extensive growth has been observed. The rate of growth of epithelial cells in vitro is less active than that of fibroblasts, but the number of cultures can be multiplied easily.

27. *Preliminary results of rotation of portions of the embryonic spinal cord about its long axis.* DAVENPORT HOOKER, University of Pittsburgh School of Medicine.

Previous studies have demonstrated the possibility of reestablishing anatomical and physiological continuity between completely severed portions of the embryonic spinal cord in normal and reversed polarity when the original dorsoventral relations of the components are maintained. The power of the various parts of the cord to reestablish proper connections in cases of poor apposition of the cut ends led to experiments on the reestablishment of continuity between severed segments of the embryonic cord of *Rana* and *Amblystoma* when one segment was rotated on its long axis.

The results of preliminary experiments indicate that it is possible to obtain reestablishment of continuity between a normally oriented cord component and a segment which has been rotated on its long axis through various arcs up to complete inversion ( $180^\circ$  rotation). The restoration of physiological continuity has not been conclusively demonstrated. Better healing and continuity of like areas of the cord is obtained when the segment is rotated up to  $90^\circ$  than when rotated more. When rotated  $90^\circ$ , there is a tendency for the regenerated tissue closing the cord wound to rotate through the quarter turn of a spiral to link up the like cord areas. This same tendency is present in rotations of from  $90^\circ$  to  $135^\circ$ , but is not so clearly demonstrable. In at least one case of  $180^\circ$  rotation, there is definite evidence of a motor and sensory fiber decussation in the sagittal plane. There is thus a definite interattraction of like areas of the cord.

28. *A note on the ectodermal origin of the spinal ganglia.* DAVENPORT HOOKER, University of Pittsburgh School of Medicine.

The results of the writer's experiments in total removal of the nervous system in frog embryos at the time of closing of the neural folds have been criticized by Dart and Shellshear on the ground that the operation necessitated the removal of the dorsal portions of the myotomes from which, according to these investigators, the cells of the spinal cord and ganglia are at least in part derived.

It is practically impossible to remove the entire nervous system in frog embryos at the stage in which the neural folds have just closed without some injury to the myotomes. It is possible to remove the dorsal half of the spinal cord, the neural crest and the covering ectoderm at this stage without the slightest injury to the myotomes. This was done, in the belief that the absence of all nervous elements in the total removal experiments in conjunction with the presence of the majority of myotomic elements proved the ectodermal origin of the ventral horn cells and made it necessary to review only the possible myotomic origin of spinal ganglia elements.

The results of the removal of the dorsal half of the cord, of the neural crest and the ectoderm show that the amount removed is in direct proportion to the size of the spinal ganglia developing, if any. Where little was removed, the ganglia are complete; where the cut went deeper, they are smaller; and where the entire mass was removed, they are absent. The myotomes contribute no elements.



29. *The relation of the thyroid to certain stages of metamorphosis in frog larvae.*

MARGARET MORRIS HOSKINS, University of Arkansas.

The experiments described were made by E. R. Hoskins in 1919, in order to test a theory of the relation of the thyroid to different stages of metamorphosis. It was our belief that the influence of this gland on metamorphosis was through its effect on calcium metabolism. In its absence the skeletal changes do not occur and the lack of these first steps towards the adult form prevents the later changes such as the atrophy of the tail and the shortening of the intestine. In the experiments made in 1919, the thyroid anlage was taken from its normal position and grafted into the tails of the larvae. When the animals had reached their maximum size and had well-developed legs, the tails containing the thyroid grafts were cut off. With few exceptions these thyroidless animals completed metamorphosis. Examination of sections has been made to eliminate all cases in which thyroids regenerated in the normal position, and it was found that although a large percentage of regeneration was present, a number of the frogs were actually thyroidless. It is therefore evident that the thyroid is not directly necessary for the later stages of metamorphosis.

30. *Meningeal relations of hypophysis cerebri.* WALTER HUGHSON, Department of Anatomy, Johns Hopkins Medical School.

Although many workers have commented upon the meningeal investments of the hypophysis, no adequate description of these membranes has been presented. Study of serial sections of this region shows that in addition to the dural covering of the floor of the sella turcica, the hypophysis is completely surrounded by an arachnoid membrane continuous with that of the brain. Similarly the pia is reflected upon the hypophysis investing it closely. Between these two membranes is a space, traversed by many trabeculae so that it becomes close-meshed.

The continuity of this leptomeningeal space about the hypophysis with the cranial subarachnoid space has been demonstrated by two methods of injection. In both of these procedures, injections of a true solution of iron ammonium citrate and sodium ferrocyanide were made into the spinal or bulbar subarachnoid space, with subsequent precipitation of the foreign salts in situ. The first method involved the injection of the solution under rather high pressures; in the second the introduction of foreign solution was accomplished by the reduction of the cerebrospinal fluid pressure by simultaneous intravenous injection of strongly hypertonic solutions of sodium chloride. Under these conditions of injection the precipitated prussian blue granules have been found to surround the hypophysis in a definitive subarachnoid space. In addition these granules may be traced into the substance of the hypophysis itself, entering by way of perivascular channels into the stroma of the pars buccalis and outlining more or less completely the cellular cords. Likewise into the substance of the pars nervosa granules may be similarly traced. In both cases, absorption into the capillary bed seems indicated.

31. *Observations on the femur.* N. W. INGALLS and M. H. GROSSBERG, Laboratory of Anatomy, Western Reserve University.

The possession by the Hamann Museum of a pair of femora of great interest and extreme rarity has directed attention to certain variations in the distal

half of the femoral diaphysis. The femora in question are characterized by their great length and especially by the very marked increase in the transverse diameter of the lower part of the shaft. While these specimens are frankly pathological, the evidence from normal bones seems to indicate that the condition just noted is only a very aggravated case of normal growth disturbance, although the etiological factors may not be the same. A rather extended series of measurements carried out on 100 pairs of femora from male whites between the ages of 18 and 86 shows, among other things, considerable variation in the width of the inferior half of the femoral shaft. Although none of the bones of this series approach in degree of deformity the pair which occasioned the investigation, they show earlier and milder stages of what appears to be a similar process, i.e., an increase in the width of the lower half of the shaft. In well marked cases there is a gradual increase in transverse diameter beginning about the center of the diaphysis, the lower half of the bone is unusually massive and the condyles consequently appear less prominent. The normal processes of growth and remodeling in this part of the bone seem to be especially sensitive or vulnerable. Somewhat similar, but more irregular deformities in this region have been supposed to be of endocrine, possibly thyroid, origin.

*32. Changes in body length and in weights of the body and of various organs in atrophic infants.* C. M. JACKSON, Institute of Anatomy, University of Minnesota.

Clinical history and autopsy data for twelve cases of death from inanition were used. Ages range from 5 to 255 days; final body weights, 1695 to 3972 grams; body lengths, 47 to 64 cm. In most cases, the data are fairly complete and comparison (using Scammon's norms) permits the following conclusions:

1. The height-weight index (metric) averages 0.0165, range 0.0120-0.0205 (Bardeen's normal, 0.254).

2. The loss in body weight, final compared with maximum recorded during life, averages 19.2 per cent (range 13.7 to 25.5 per cent).

3. The loss in body weight, final compared with normal for corresponding body length, averages 28.5 per cent (range 7.4 to 52.3 per cent). The loss estimated on this basis is greater, due to persistent skeletal growth.

4. The retardation in growth, final body weight being compared with normal for corresponding age, averages 56.8 per cent (range 41.6 to 71.6 per cent).

5. The changes in weights of the individual organs have been calculated, each observed weight being compared with the normals estimated as the corresponding values for, 1) the final body weight; 2) the maximum body weight; 3) the final body length, and, 4) the age. In general the normal weights are progressively greater for these four estimates, with corresponding differences in the relative (percentage) changes in the organ weights.

6. Omitting consideration of the individual organs, it may be stated that the results are in general agreement with those obtained by experimental inanition on animals. The differences may be due partly to the small number of cases and to existing complications in the atrophic infants.

33. *Secretory phenomena in pathological human thyroids.* J. ALBERT KEY, Harvard Medical School.

In hyperactive thyroids the mitochondria and secretion antecedents are increased and can be studied. In a series of fifty pathological thyroids I have studied the mitochondria and secretion granules and vacuoles.

The secretion granules, first differentiated from the mitochondria by Bensley, are round, variable in size and usually in the apical zone of the cell. They are soluble in alcohol, blackened with osmic acid, and are fixed and stained by mitochondrial techniques. They differ from mitochondria in that they are more resistant to acetic acid and are stained by methyl-green, neutral gentian and other stains which do not stain mitochondria.

The secretion vacuoles first seen by Wyss, and termed chromophobe secretion by Anderson, are apparently identical with those described by Bensley. They are variable in size, most abundant in the apical zone of the cell, and appear as clear vacuoles in formalin-bichromate or osmic material. By Bensley's formalin-zenker and brazilin-wasserblau method the vacuoles deep in the protoplasm are preserved and contain a thin coagulum which stains light blue, but the ones in the apical zone are destroyed.

The mode of secretion seems to be that the granules and vacuoles are cast into the vesicle and lose their identity, the granules being dissolved in the fluid like contents of the vacuoles and the mixture forming the colloid which passes back between the cells to be taken up by the lymphatics or blood vessels. The density of the colloid is determined by the relative proportion of the two constituents. The vacuoles are markedly increased in the hyperactive glands and cause the colloid to be thin and contain large vacuoles.

The granules may or may not be increased and their rôle is apparently to form a thickening menstruum to hold the active constituent (contained in the vacuoles) in the vesicles. The mitochondria are definitely increased in hyperactive glands but apparently have to do with the general metabolism of the cell and are not the direct antecedents of the secretion. All three appear to arise independently in the cytoplasm. The colloid cells are senile cells and intracellular colloid droplets are evidence of hypofunction.

34. *Peripheral migration and distribution of cells from the cerebrospinal nervous system in embryos.* ALBERT KUNTZ, St. Louis University School of Medicine.

In embryos of the chick in which the neural crests and the dorsal portions of the neural tube are destroyed by early operation the primordia of the sympathetic trunks arise exclusively from cells of medullary origin which advance peripherally along the paths of the ventral roots of the spinal nerves. Migrant medullary cells also become incorporated in the neurilemma of efferent nerve-fibers. These facts were demonstrated at the last meeting of the American Association of Anatomists.

Further experimental studies indicate that the majority of the cells which become incorporated in the primordia of the sympathetic trunks advance from the intermediate portions of the lateral walls of the neural tube.

In embryos in which the peripheral migration of cells from the cerebrospinal nervous system is prevented throughout the trunk region by early operation the primordia of the oesophageal, pulmonary, cardiac, and enteric plexuses arise

exclusively from cells which advance peripherally along the paths of the vagi. On the other hand, an embryo in which the portions of the hind-brain from which the vagi arise and the greater portion of the vagus ganglia were destroyed by early operation shows complete absence of the primordia of the sympathetic plexuses related to the vagi although the primordia of the sympathetic trunks and the prevertebral plexuses are present.

35. *The innervation of the lung of the rabbit.* O. LARSELL, University of Oregon Medical School.

The bronchial tubes are innervated chiefly by the homolateral vagus, but fibers from the contralateral nerve also have a part in each lung.

The larger bronchi are surrounded by two nerve plexuses. One, composed of relatively large fibers, many myelinated, lies external to the cartilaginous plates. Numerous clusters of ganglionic cells are distributed throughout this plexus. Unmyelinated axonic processes from these cells become arranged into bundles of small size which form a second plexus between the bronchial muscle and the plates of cartilage. The individual fibers subdivide and terminate on the smooth muscle cells. Frequently accompanying these bundles are larger myelinated fibers which lead to sensory terminations in the bronchial epithelium.

In the smaller bronchi the two plexuses become intermingled so closely that only one may be said to be present. Relatively few ganglion cells are found beyond the secondary bronchi, so that the plexus of the smaller bronchi consists chiefly of postganglionic fibers and sensory fibers, whereas the extracartilaginous plexus of the larger bronchi consists chiefly of preganglionic fibers and sensory fibers.

The pulmonary blood-vessels, including the bronchial artery, have a well defined plexus of nerve fibers which terminate in the smooth muscle cells of the tunica media of these vessels.

Myelinated nerve fibers and small nerve terminations, apparently of sensory type, are also present in the pulmonary pleura.

36. *Empirical formulae for the growth curves of the various organs and systems of the single-comb White Leghorn chicken.* H. B. LATIMER, University of Nebraska.

Empirical formulae for the growth curves of one hundred single-comb White Leghorn chickens ranging in age from day of hatching to three hundred days, with six between one and two years old, have the general formula;  $Y = (aX)^n \pm bX \pm c$ .  $Y$  represents the weight of the organ, in grams;  $a$ ,  $b$ ,  $c$  and  $n$  are fixed constants, empirically determined, and  $X$  represents the gross body weight in grams. The entire growth curve for some of the organs may be represented by this formula with slight variations of the constants for each curve, and at least the first part of all the curves can be so expressed.

For the organs with a curve which is convex superiorly at first " $n$ " is a decimal fraction. This group includes most of the organs such as the brain, spinal cord, eyeballs, kidneys, etc. In the formula for the thyroid gland, feathers and musculature " $n$ " is greater than unity and the resulting curve is concave superiorly in at least the first part of the curve. The formula for the growth of the integument (without feathers) and for the ligamentous skeleton is a straight line at first.

The upper or terminal portion of many of the curves is a straight line, represented by the general formula  $Y = a(X - b) + c$ , in which  $a$ ,  $b$  and  $c$  are the constants and  $X$  the gross body weight.

A sex difference is shown in the growth curve of the feathers and the ligamentous skeleton.

37. *Studies on the retina. Histogenesis of the visual cells in Amblystoma.* HENRY LAURENS and SAMUEL RANDALL DETWILER, Yale University and Pekin Union Medical College.

The first stage in the development of the visual cell is the production of a protoplasmic bud and a clear achromatic globule from the cells of the external nuclear layer. The globule, which becomes the paraboloid of the inner segment, appears to be of cytoplasmic origin. In early stages of the visual cells, masses of deeply staining granules are seen in iron-haematoxylin preparations. These granules contribute mainly to the formation of the ellipsoid of the inner segment and to the granular material of the outer segment. There is no evidence from our preparations which indicates that they are products of pigment ingested from the epithelial layer. They may represent transformation products of mitochondria. In early stages of development, the visual cells are all cone-like, agreeing in this respect with the conditions characteristic of the developing amphibian retina. Rods and cones are later differentiated from the primitive, non-specialized visual cells. Rods do not appear in their definitive form until relatively late. The two kinds of conical-shaped visual elements are regarded as visual cells of low specialization which develop, respectively, into characteristic rods and cones by divergent differentiation.

38. *Studies on the ligation of the thoracic duct.* F. C. LEE (introduced by Lewis H. Weed), Department of Anatomy, Johns Hopkins Medical School.

a. *The establishment of collateral circulation.* An operation was devised for the intrathoracic ligation of the thoracic duct in the cat. Subsequent injection of the mesenteric lymphatic vessels while the animal was under an anesthetic showed that two types of collateral circulation were established. One group consisted of those cases in which branches came off of the thoracic duct below its point of ligation and then joined the right thoracic duct. In one animal this connection was seen as a fine branch seven days following ligation; while in another animal which was sacrificed seventy-seven days after ligation, two large trunks coursed to the right thoracic duct. The other group included those cases in which a lymphatico-venous connection was established between the thoracic duct and the azygos vein or its branches. b. *Changes in the number of small lymphocytes in the circulating blood.* Blood counts and smears were made from a series of animals in which the duct had been tied, and it was found that the absolute number of small lymphocytes decreased about sixty per cent immediately following ligation, but that the number gradually returned to normal at the end of three weeks. Examination showed that a collateral circulation had been established.

39. *A. On the influence of symmetry in development, with special reference to the aortic arches of the sheep. B. Lantern slides to illustrate the derivation of the term 'hippocampus,' with preparations by Prof. P. E. Lineback.* FREDERIC T. LEWIS, Harvard Medical School.

'Nature abhors a vacuum' is a discredited medieval maxim, serviceable as it was in its day. With similar limitations it may be said that Nature is ill at ease—restless—in the presence of asymmetry, thus expressing a milder reaction, subsiding into apparent contentment when conditions are irremediable. Two examples of this situation, one from plants and one from animals, will be presented. The flowers of *Campanula rapunculoides*, with parts in fives except the carpels, which are three, show many variations interpretable as readjustments. Frequently by the addition of a sixth sepal, petal, and stamen symmetry is restored. A most striking instance in mammalian embryos is the almost complete restoration of a new symmetry, after the first has been destroyed, in the aortic arches of the sheep. The transformation of these vessels has been studied by Dr. G. H. Jackson, Jr., whose results, in part, I am permitted to report. Wax reconstructions of embryos of 10, 14, 15.8, 18, and 47.5 mm. have been made.

If the attainment of symmetry may be regarded as an end in nature, subsequent changes in the great vessels in the pig would seem possible, and the human aortic arches are perhaps further from their ultimate arrangement than those of either pigs or sheep. Such considerations, at least, make it possible to suggest morphological changes in the development of the human body, which, in the course of time, may yet be brought about.

40. *Is mesenchyme or smooth muscle a syncytium or an adherent reticulum?* WARREN H. LEWIS, Carnegie Laboratory of Embryology.

Conclusive evidence on this point cannot be gained from fixed, stained and sectioned material; on the other hand, cultures indicate that the cells of these tissues unite not as a syncytium, but as an adherent reticulum, often similar to that seen in the embryo. One can follow the slow shifting of the cells, the withdrawal of processes from neighboring cells and the sending out of new ones to the same or other cells. Processes attached to the cover-glass only, behave the same as do those attached to neighboring cells except that the former are often more firmly adherent. The processes of mesenchyme cells tend to extend out in all directions and can often be followed in outline (even in fixed cultures) onto neighboring cells or cells beyond these. The processes of smooth muscle cells are usually longer and more pronounced at the two poles and may extend for long distances. There is no evidence of the transfer of material from one cell to another. Both types of cells often become entirely isolated at the periphery of the outgrowths from the rest of the reticulum, their processes being attached solely to the cover-glass. Hypertonic solutions, glycerin and other reagents may cause cells of a reticulum to round up and lose all connections with neighboring cells. In one type of degeneration a similar effect is produced. A renewal of the favorable medium may cause the cells again to expand into an adherent reticulum. Daughter cells do not remain permanently attached to each other.

41. *Survival of cells after death of the animal.* WARREN H. LEWIS and C. C. McCox, Johns Hopkins Medical School.

All cells live for a period after death. Most of them develop granules and vacuoles which have an affinity for neutral red. When the cells die the color disappears from the granules and vacuoles and the nuclear wall becomes marked. With neutral red as a testing agent various tissues of the rat were examined in spreads. In organs kept at 37°F. large macrophages, cartilage cells, kidney epithelium and smooth muscle, survived for 240 hours; bladder, trachea, tongue and salivary gland epithelium, 192 hours; lymphoid cells and endothelium, 168 hours; lung epithelium, 144 hours; Kupffer cells and leucocytes, 120 hours; sertoli, liver and pancreatic cells and brain macrophages, 96 hours; mesenchyme, ovarian follicular, uterine epithelial and gland cells and red blood corpuscles, 72 hours; and epididymis epithelium, 48 hours. Small pieces of tissue left at room temperature in Locke's solution with neutral red exhibited living macrophages at 144 hours; bladder, tracheal and salivary gland epithelium, smooth muscle and cartilage cells, at 120 hours; mesenchyme and endothelium at 72 hours; ectoderm, lymphoid cells, uterine, pancreatic and lung epithelium, at 48 hours; adrenal cells and leucocytes, at 24 hours; epididymis and intestinal epithelium, ovarian follicular, liver and fat cells, at 18 hours; red blood corpuscles, male germ cells and sertoli cells, at 12 hours. When the entire animal was left at room temperature the periods of survival were much shorter. Nerve cells usually die before granules and vacuoles have a chance to form. Striated muscle always stains diffuse pink.

42. *Studies on the nerve supply to the colon: First showing in the early stages with a model of a 23-mm. embryo.* P. E. LINEBACK, Emory University, School of Medicine.

A study of the appearance and early nature of the nerve supply to the colon, following closely the work done on its musculature, seems logical. This is all the more advisable since the study of both the nerves and muscles in the older, fuller formed state will be greatly facilitated by working with the two simultaneously.

Knowledge of the muscle-nerve mechanism of the colon is of a general sort; it being commonly accepted that the nerves are entirely derived from the sympathetic system. Lynch and Draper have suggested that there might be some degree of nerve control in the caecal end of the tube derived from the mesenteric plexuses, hence a vagus admixture.

In conjunction with the above unsettled point there is the problem of the detailed distribution of the nerves to the sacculi and taeniae. It has been shown that independent segmental contraction occurs in the three rows of sacculi and it is reasonable to infer that there is an independent nerve supply to each. Such details are lacking in the literature and it is the purpose of the author to pursue this line of investigation, beginning with the earliest appearance of nerves in the embryo.

As shown by the 23-mm. model two large nerves grow out from the hypogastric plexus and extend along the tube, forward. These are more prominent and advanced than those from the mesenteric plexuses in the caecal end. This is in harmony with the origin and line of growth of the muscles.

43. *The effect of the ablation of the superior cervical sympathetic ganglia upon the continuance of life.* M. LAURENCE MONTGOMERY (introduced by P. E. Smith), Department of Anatomy, University of California.

In a recent paper Meltzer has stated that the cat and rabbit usually die within a few days if both superior cervical sympathetic ganglia are ablated. Evidence derived from transplantations inclined this author to believe that these ganglia elaborate a principle peculiar to themselves and which is essential to the life of the animal. I have tested this point in a total of eighteen animals, in the rat, cat, and rabbit. All my animals, save one cat, survived the ablation of these ganglia. The cats and rabbits were tested for the presence or absence of these bodies by the 'paradoxical' eye reflex of Meltzer. It is true that the majority of such animals give this reaction. At the autopsy performed when the animals were sacrificed fourteen to sixty-eight days after the operation the tissue at the site of operation was removed, later being serially sectioned and examined histologically. No superior sympathetic ganglionic tissue was present in most cases. This shows that these bodies are not essential to life in these animals and that they do not elaborate an essential secretion peculiar to themselves.

44. *A preliminary report on a radiographic study of the position of the stomach and other abdominal viscera in healthy young adults.* ROBERT ORTON MOODY and R. G. VAN NUYS, University of California, and W. E. CHAMBERLAIN, Leland Stanford Junior University.

This study of three hundred students, an equal number of men and women, shows that in 90 per cent of the women and 81 per cent of the men, in the erect posture, the most caudal part of the greater curvature of the stomach is caudad to a line connecting the highest part of the iliac crests (interiliac line).

The most cephalic position found of this part of the greater curvature is 6 cm. cephalad of the interiliac line and the most caudal position 12 cm. caudad to this line. Dividing this distance into quarters, the position of the most caudal part of the greater curvature is shown in the following table, + indicating the distance in centimeters cephalad and - indicating the distance caudad to the interiliac line.

	+ 6 to + 1.5		+ 1.4 to - 3		- 3.1 to - 7.5		- 7.6 to - 12	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Males.....	16	10	53	35	60	40	21	14
Females..	9	6	45	30	58	38	38	25
Total...	25	8	98	32	118	39	59	19

These results indicate that a low position of the stomach is normal in young adults. It is not ptosis and is not a frequent cause of poor health.

It is also found that, contrary to common belief, the strength of the ventral abdominal musculature and the depth of the lumbar curve are not significant factors in the determination of the position of the stomach.



This problem includes a study of the physical factors that may modify the position of the abdominal viscera. A report on additional cases will be made later.

45. *Studies of the estrous cycle in the ox.* H. S. MURPHEY, Iowa State College.

A. Diestrurn: Os circular, vagina comparatively dry, very few epithelial cells, practically no leucocytes.

B. Estrum: Pro-estrurn: 1. Bright cherry-red color of vestibule due to congestion of bulbus vestibuli. 2. Enlargement of vaginal part of cervix due to swelling of or ripening of mucous cells. 3. Beginning of the outpouring of mucus from the cervical canal. 4. Slight swelling of the vulva. Estrum (or heat): 1. Signs:—bawling, uneasiness, depression of lumbo-sacral articulation; on digital manipulation of clitoris, receptive movements. 2. Swelling of vulva. 3. Vestibule deep red to mahogany. 4. Involuntary contractions of vestibular sphincter. 5. Os uteri, a transverse slit. 6. Great swelling and protrusion into the vagina of the folds of mucous membrane of the cervix. The folding is more marked, and arranged in three to four rows—in old cows mostly two encircling rings—while in virgin heifers mostly one and a half rings. 7. A marked outpouring of clear, viscid, cohesive mucus.

Post-estrurn: 1. A sudden cessation of estrum or heat manifestations. 2. A gradual cessation of the anatomical changes. The vulva becomes wrinkled. The deep color of the vestibule remains relatively longer; the enlargement of the cervix gradually subsides; mucus is discharged from the os for two or three days. The vaginal mucus is streaked with or shows white flocculi, later it becomes gummy, sticky and cohesive, it is opalescent. The white material is both epithelial cells and leucocytes. The former are vaginal in origin as are most of the leucocytes; however, some of the latter are present in smears made directly from the os. Microscopically the cells show increasing degeneration step by step with the gumminess of the mucus.

Diestrum:

Estrum: Uterus: 1. Marked congestion. 2. Edematous, glassy, mucous membrane folded in hillock fashion. 3. Petechiae on both the cotyledonous and intercotyledonary mucous membrane.

46. *Dorsal and ventral implantation of the limb bud.* J. S. NICHOLAS, University of Pittsburgh School of Medicine. (From the Osborn Zoological Laboratory, Yale University, and the University of Pittsburgh.)

The limb bud has been transplanted to the ventral and dorsal midlines in order to test the rules of limb asymmetry. The growth of the limb so implanted is seldom typical and the limb is generally resorbed.

The two cases in which limbs developed, one in the ventral and one in the dorsal location, gave rise to two perfect limbs. These cases are in accord with Harrison's rules for limb behavior, for under the conditions of implantation the limb bud was located half on the right and half on the left side of the animal. The limbs are morphologically complete in every respect. The girdles of the two limbs are fused, the central portion being common to both members.

The ventral limbs are innervated apparently by the rami supplying the ventro-lateral musculature. There is no increase in the peripheral distribution of the

normal nerves from the plexuses of the normal limbs. Below the dorsally implanted limbs numerous mitoses are found in the cord which shows definite hyperplasia. The sensory ganglia are found dorsal to their regular location. This hyperplasia and displacement of the sensory ganglia cannot be said to be due to operative defect, for other animals in which the limb resorbed were operated in the same manner but no hyperplasia exists in any of the cases so far examined. It may be assumed that there has been a definite influence exerted by the limbs developing in the dorsal location which has caused hyperplasia.

47. *The effect of the rotation of the area surrounding the limb bud.* J. S. NICHOLAS, University of Pittsburgh School of Medicine. (From the Osborn Zoological Laboratory, Yale University, and the University of Pittsburgh.)

Since it has been shown that the limb bud tends to recover its normal posture during its development after the rotation of the embryonic rudiment, the next step is to locate the factors which take part in the process of recovery.

By using a double operation with the rotation of the area surrounding the limb bud, the relationships of both the normal limb area and the surrounding portions have been tested. The complete operative disc is five somites in diameter, of which the central three and one-half somites are occupied by the limb bud.

The results of these operations show that in the course of development, the area surrounding the limb bud controls the postural relationship of the developing limb. The limbs produced are normal except for the posture which they assume. When the limb bud is oriented normally with reference to the embryo, but the surrounding tissue is rotated, the limb is rotated during its development so that it assumes its posture with reference to the surrounding area and not to the organism as a whole. This occurs in both the orthotopic and heterotopic locations, thus showing that the factors causing the rotation of the limb are located in the area normally surrounding the limb.

Further experiments will be carried out to show definitely the development of the parts which are located in the area surrounding the limb and also to show the specific influence of the individual components during the course of their development.

48. *An anthropometric study of a group of Syrians in the United States.* G. J. NOBACK, Medical College of Virginia.

This study was made with the object of determining the physical characteristics of one of the peoples of recent migration to the United States. The lack of this information regarding the Syrians led to this study in Minneapolis and St. Paul. One hundred and thirty individuals were measured, of which ninety-four were male and thirty-six were females. Their ages ranged from sixteen to sixty-five years.

The technic and measurements follow closely those practiced in the anthropometric work of the Smithsonian Institution (Hrdlicka, '21). Twenty-two measurements and ten other observations were made on each individual, and ten indices were determined.

Most of the individuals were from the Lebanon and were from mountain and plain. It is believed that our conclusions apply to all of the middle third of Syria.

In stature this group of people is medium-tall according to the classification of heights by Martin and are 2.7 above the world average of Bean. The skeletal index is 52.9; i.e., 2.9 above the index for the world. The cephalic index is 87.5; i.e., Brachcephalic and 7.5 above the world average of Bean. The nose is Leptorhine, index being 65.4, and 14.6 less than world average of Bean. The facial index is 91.5; i.e., Leptoprosopic. The nose is straight or slightly convex in 94 per cent of the cases; the hair color is brunette in all but one (pure blond) case; the eyes predominantly brown though there are four pure blue and eighteen with some degree of blueness.

The teeth show a poorer condition in those born in the United States on comparison with a similar age group of those born in Syria.

49. *On the ossification of the cartilages of the human larynx.* G. J. NOBACK, The Medical College of Virginia.

Previous to Chievitz ('82) it was believed that ossification of these cartilages did not occur before middle life. Investigators were divided as to its significance. Some believed it a normal development, others that it had some relation to disease. Chievitz stated that it was a normal process and that ossification of the laryngeal cartilages is present in all males over twenty, and in all females over twenty-two years of age. Scheier ('01) states that it is a normal process beginning at the same time in both sexes and that he observed ossification in a girl nineteen years old. Text-books follow Chievitz mainly, though some state that this ossification appears at twenty-five years and others that it appears after middle age.

Material used in this study consists of 34 cadaver larynges cleared with KOH and 13 cadaver larynges of which x-rays were made, x-rays of the cervical region of 33 individuals in normal health, and x-rays of 48 persons with chronic tuberculosis.

The present study shows that ossification in the laryngeal cartilages may occur earlier than previously believed. It is demonstrable in the x-ray picture of a boy of seventeen years and in that of a girl of the same age.

Since those individuals showing earliest ossification have chronic tuberculosis and since individuals with this disease show more frequent and extensive ossification of the laryngeal cartilages it seems that chronic diseases may have an intimate relation to ossification of these cartilages.

50. *Nerve supply of atria of the bovine heart.* JAMES W. PAPEZ, Cornell University.

The bovine atria are supplied by the right and left vago-sympathetic nerves.

The right atrial nerves, three in number, enter along the left wall of the superior vena cava. 1) The largest branch enters the triangular fossa in the atrial septum dorsal to the interatrial band and divides into two. The ventral one (interatrial n.) has a small enlargement from which several filaments are given to the interatrial band, two passing to the left end and one to the right end of the band. The dorsal one (septal n.) has a small enlargement which gives filaments to muscle bundles that radiate from the septum into the walls of both atria. 2) The dorsal branch (right sinus n.) supplies filaments to the musculature of the superior vena cava and in the intercaval region it has an enlargement which gives filaments to the intercaval bundle and muscle bundles around the orifice of the

inferior vena cava. 3) A communicating branch passes dorsal to the right pulmonary veins to form a loop with the left sinus nerve which gives filaments to the dorsal wall of the left atrium and ventricle.

The left atrial nerves enter along the left horn of the sinus venosus (left superior vena cava). 1) The ventral branch has an enlargement at the base of the atrial appendage which distributes filaments to the musculature around the base of the appendage and toward the septum. 2) The lateral branch is distributed to the left and dorsal surface of the atrium. 3) The dorsal branch is distributed along the wall of the left horn of the sinus and communicates with a loop of the right nerve.

51. *Structural factors contributing to acoustic insulation of the end organ.* A. G. POHLMAN, St. Louis University.

The diaphragm-rod theory of sound transmission as presented in part at the past two meetings is opposed to the commonly accepted physiology of the middle and inner ear complexes. The usual theories assume a displacement factor in the perilymph as the activating agent, whereas the writer contends for a direct activation of the auditory cells by the sound pulse itself. Accordingly, the conception of end organ response demands an interpretation on the basis of sound pulse activation. Evidence will be submitted which points to the following: First, that the sound pulse enters the scala vestibuli through diffraction. Second, that the diffracted sound pulse is damped by the relatively small opening through which it passes from the vestibule. Third, that the spiral course of the cochlear structure further limits the amount of energy reaching the region of the helicotrema. Fourth, that the membrana basilaris acts in part as an insulator against the discharge of sound pulses from the otic capsule into the end organ. Fifth, that the vestibular membrane tends to limit the amount of energy transmitted from the scala vestibuli into the cochlear duct.

This conception therefore assumes that the auditory epithelium occupies a relatively sound-proof area except to energies entering through the footplate. It also takes into account the considerably greater energies required to hear the low-pitch tones. It explains the failure of acoustic insulation against high-pitch tones of considerable intensity.

52. *The effects of ultraviolet rays on developing mollusks (Limnaeus).* C. W. M. POYNTER, University of Nebraska, Omaha.

A mercury vapor lamp was used and the apparatus so arranged that the light came from below which permitted the free use of the microscope for continuous observation. Pond snails are particularly adapted for this type of investigation not only on account of their penetrability and excellent visibility but also because their early motility and later heart beat make it possible to study the early effects of sub-lethal doses of the ray.

It must be kept in mind that embryos are more sensitive to the rays at some periods of development than at others if quantitative observations are to be made. From the study of the lethal effects of the rays on a large number of embryos of the same age it was found that the range of resistance within the group was greater than the resistance of the weakest members.

A comparison of short non-lethal doses with continuous cytotoxic doses suggests that one of the effects of the light is to form a phototoxic substance which is cumulative in its effects. These animals do not show a greater heat sensitivity from raying; although they die when placed in higher temperatures it can be shown that it is not because of a specific heat action. The effect of the rays in small doses is frequently spoken of as stimulating, but in this work it seems quite certain that the stimulus is catabolic in character. These animals do not present the same recovery curve reported for parameciae. Very small doses of the ray produce a wide variety of monsters.

*53. Differences in the time of developments of centers of ossification in the male and female skeleton.* J. W. PRYOR, University of Kentucky.

"The bones of the female ossify in advance of the male. This is measured at first by days, then months, then years" (American Journal of Anatomy, 1907). The above observation was made after the study of five hundred and fifty-four roentgenograms of the hands of children from three months to fourteen years of age. Two hundred of these were under seven years of age. The conclusion was based on the development of centers of ossification in the bones of the carpus; in the epiphyses of the metacarpals, phalanges, and the distal extremity of the radius and ulna. Comparisons were made between brother and sister; twins of the same sex; twins of the opposite sex and the average of male and female hands of the same age. With these findings in view and the conclusions accurate, the first centers of ossification appearing in the embryo would be in the female.

Mall states the first centers of ossification develop in the clavicle at the sixth week. He did not make a distinction between the male and female. It is probable these early centers were in the female. The conclusions in this paper are based upon the study of roentgenograms of one hundred and forty foetuses from ten and one-half weeks to thirty-eight weeks of age, of which seventy-one were male and sixty-nine were female; and one hundred new born babes from a few hours to ten days of age, of which forty-eight were male and fifty-two were female. Very few of the foetuses were under twelve weeks and the early centers could not be compared. The principal points to be compared were the centers of ossification of the calcaneum, the talus, the cuboid in the tarsus, the epiphyses of the distal extremity of the femur, the central extremity of the tibia and centers of ossification of the capitatum and hamatum of the carpus.

*54. The distribution of acid cells on the dorsal wall of the stomach.* H. E. RADASCH, Jefferson Medical College.

In a previous paper the distribution of acid cells along the greater, lesser and ventral curvatures was carefully worked out. At that time the distribution along the dorsal curvature was not recorded. Thinking that the distribution here might afford some connection with the more prevalent occurrence of gastric ulcer upon the dorsal wall, a special study was made of the distribution of acid cells here.

The average distance along the lesser curvature (from the cardiac orifice) of all human stomachs studied is 63.09 per cent. The average distance along the greater curvature of all human stomachs is 83.29 per cent. The average distance along the ventral curvature is 76.45 per cent. In reference to the dorsal curva-

ture, the average distance from the cardiac orifice that the acid cells extended was 82.76 per cent.

In comparing these distances, it will be seen that the acid cells extend farther along the greater curvature than along the ventral or dorsal curvatures, while the acid cells do not extend along the dorsal curvature to quite the same extent as along the ventral curvature. The areas along these curvatures at which the acid cells cease represent a plane that is not set at a right angle to the axis of the gastric canal, but slopes from the lesser curvature caudad and to the left; the plane as a whole faces cephalad and slightly dorsad.

55. *Antidromic conduction considered anatomically.* S. W. RANSON, Northwestern University Medical School.

Bayliss has shown that the vasodilator fibers for the limbs run through the dorsal roots and have their cells of origin in the spinal ganglia. He believes that they are sensory fibers with side branches terminating on the blood vessels, and that these fibers conduct afferent impulses centrally and vasodilator impulses antidromically. This conception has received strong support from the work of Bruce, Bardy, Richardson and Wyatt, Januschka, and Krogh. But it has not been proven that the dilator impulses leave the spinal cord by way of the sensory fibers of the dorsal roots and it must not be overlooked that fibers of unknown origin and function enter the spinal ganglia and terminate about the spinal ganglion cells in pericellular plexuses which are practically identical with those found in sympathetic ganglia. It is quite possible that the pericellular plexuses represent the synapses between preganglionic and postganglionic vasodilator neurons.

The theory of antidromic conduction as stated by Bayliss does not allow for any synapse of sympathetic character; but it can easily be shown by the use of nicotine that there is such a synaptic interruption in the vasodilator path. Plethysmographic tracings taken from the hind leg of the cat show that the vasodilator reflex which can normally be obtained by central stimulation of the vagus nerve is eliminated by suitable doses of nicotine. Whether this interruption occurs in the spinal ganglion or at some other point remains to be determined.

56. *On the time and mode of transition from the fetal to the postnatal phase of growth in man.* (Lantern.) RICHARD E. SCAMMON, Department of Anatomy, University of Minnesota.

The growth of the human body exhibits quite different characteristics in the fetal and the postnatal divisions of the developmental period. The results of a quantitative study of the transition between these phases indicate that the changes which occur may be grouped in two quite distinct classes. The first are the true natal changes associated directly with the shift from intrauterine to extrauterine life. These include modifications in the suprarenals, uterus, stomach, lymphoid tissues and lungs, and the postnatal weight loss and recovery. These changes have certain characters in common: they occur immediately after birth, are of short duration, and take place regardless of the age of the fetus when born. Despite these natal changes the growth of the body as a whole appears little affected by birth and the general course of prenatal growth con-

tinues for some time thereafter. This is demonstrated by the projection by formulae of fetal growth curves into postnatal life and their comparison with observed findings for infants in the same period. It is also indicated by the study of the weight increment curves of premature children in early infancy. The true transition from the fetal to the postnatal course of growth takes place through a second series of changes, much more gradual than the natal changes, which occur mainly in the third trimester of the first year of postnatal life.

57. *A quantitative study of the growth of the human eyeball and optic nerve.* RICHARD E. SCAMMON and ELLERY L. ARMSTRONG, Department of Anatomy and Department of Ophthalmology and Oto-Laryngology, University of Minnesota.

The growth in volume of the eyeball in the fetal period agrees very closely with that of the brain-stem and spinal cord. The growth of the diameters of the eyeball is also very like the lineal growth of the brain-stem and spinal cord and may be expressed by the same general empirical formula:  $Y = a [(bX)^c \pm d]$ , where  $Y$  is the dimension in question,  $X$  the body length and  $a$ ,  $b$ ,  $c$ , and  $d$  are constants. The postnatal growth of the eyeball follows more closely that of the cerebrum than that of the brain-stem or spinal cord. The postnatal growth in volume of the eyeball seems to have been distinctly overestimated; our data indicate that the postnatal gain is not over 150 per cent. The eyeball diameters increase 35 to 40 per cent in postnatal life. In the latter part of fetal life the temporal segment of the eyeball grows more rapidly than the nasal segment. The fetal growth of the diameters of the cornea is not like that of the eyeball as a whole, but resembles the growth of the external dimensions of the body. It may be expressed by the general empirical formula for external dimensions:  $Y = aX \pm b$  (symbols as in formula above). The postnatal growth of the cornea approaches more closely that of the eyeball, although it is not entirely similar. The fetal growth in length and diameter of the optic nerve bears a simple relation to the increase in body length and may be expressed by the formula just quoted.

58. *The growth of the skeleton in the fetal period as illustrated by a quantitative study of the human mandible.* R. E. SCAMMON and S. J. Z. GANZ, Department of Anatomy, University of Minnesota.

The lineal growth of the human mandible in the fetal period was determined by measurements of 14 dimensions on a series of about 100 specimens. The calculation of the changes in mass were based upon a series of observations by Corrado. These data show that the lineal dimensions of the fetal mandible increase in a direct relation to the growth in the total body length and may be expressed by the general empirical formula:  $Y = aX \pm b$ , where  $Y$  is the dimension in question,  $X$  is the body length and  $a$  and  $b$  are constants. The growth in weight of the mandible, when plotted against body length, forms a typical weight or volume curve expressed by the general empirical formula:  $Y = aX^b + c$ , where  $Y$  is mandible weight in grams,  $X$  is body length in cm., and  $a$ ,  $b$ , and  $c$  are constants. The various angles of the mandible are extremely variable throughout the fetal period but show no marked change. Thus the mandible, despite its peculiarly intimate relation with the dental follicles, follows the simple laws which are found to be characteristic of the fetal growth of the external bodily

dimensions and of the various parts of the body. Since this holds true for the mandible and is also true for lineal dimensions taken between external bony landmarks it seems safe to conclude that the lineal growth of the skeleton and its parts in the fetal period is proportional to the lineal body growth and that the mass increase of the skeleton follows the same course as the body as a whole, its main parts and the major viscera.

59. *The genetic interpretation of abnormalities in the anatomy of the nasolacrimal passageways.* J. PARSONS SCHAEFFER, Jefferson Medical College.

From a genetic viewpoint the abnormalities in the anatomy of the nasolacrimal passageways appear to fall into three more or less clearly defined groups: First those that have their basis in rudiment potentials; second those due to early arrests in the development of parts which should proceed from the stem rudiment; and third those due to division of previously continuous parts of the nasolacrimal passageways into discontinuous segments.

Rudiment potentials are encountered which explain such abnormalities as supernumerary lacrimal ducts for one or both eyelids, duplication or triplication of the lacrimal punctum, the slit lacrimal punctum, mucosal ledges and so-called valves within the nasolacrimal passageways, irregularities of contour, diverticula, variations in the anatomy of the lacrimal aperture in the inferior nasal meatus, etc.

Patients are encountered with one or both lacrimal ducts wanting in whole or in part, the connection with the inferior nasal meatus may be defective or wanting, atresias of portions of the nasolacrimal passageways are rather common in the newborn. The cause for the failure of normal development is not clear. So far as the lacrimal ducts are concerned there appears to be a critical period for them shortly after the detachment of the solid epithelial rudiment of the nasolacrimal passageways from its surface connection. If the lacrimal ducts, which normally grow as secondary sprouts from the stem rudiment, fail to show beginning growth shortly after this stage, there appears to be a great likelihood they they will not develop subsequently.

60. *The smooth musculature of the afferent veins, and the innervation of the human suprarenal gland.* HUBERT SHEPPARD, Atlanta Medical College, Emory University.

The central adrenal vein in man contains a greater amount of smooth muscle tissue than other veins of corresponding caliber. A distribution of the muscle fibers in the afferent veins as well as in occasional accessory veins is peculiar in that most of the fibers are longitudinal and often project into the lumen of the vessels in a rugose or valve-like manner. Only a thin layer of circular fibers can usually be observed lying beneath the endothelium. Nerve plexuses in the capsule distribute to both the cortex and the medulla. Within and about the smooth musculature of the central vein, and in the other veins where smooth muscle is present, minute ganglia with occasional isolated nerve cells are found. The longitudinal fibers projecting into the lumen of the vein are abundantly supplied with minute nerve terminals. Fibrils, exceedingly fine and varicose, also penetrate between and supply the cells of the medullary epithelium. In view of the relation of epinephrin to anaphylactic shock, this abundant smooth muscu-



lature of the afferent veins in the human suprarenal as well as the delicate nerve supply in both the smooth musculature and the medulla would seem to have a direct physiological and clinical significance.

61. *Vital staining of human and mammalian blood, with special reference to the separation of the monocytes.* MIRIAM E. SIMPSON (introduced by Herbert M. Evans), Department of Anatomy, University of California.

Studies on living blood cells and vital dyes reported at the last meeting of the Association have been brought to completion. (On the reaction of the living blood cells to dyes. M. E. Simpson, Proc. Amer. Assoc. Anat., Anatomical Record, 1921, vol. 21, p. 82.) At that time it was pointed out that certain dyes (especially neutral red, Nile blue sulfate, and brilliant cresyl blue) stained a characteristic set of granules in blood cells termed the segregation granules or segregation apparatus, and furthermore that in this way the monocytes could be separated from the lymphocyte cells. The test has been applied extensively to the blood cells of experimental animals (rabbits) as well as to human material in view of the fact that the tests previously employed to separate these cells in human blood (the Giemsa stain and the oxydase reaction) may be notoriously unreliable with other mammalian blood.

The low content of lymphocytes in the segregation granules and the exceptionally high content of monocytes enables us to separate these cells. Lymphocytes possess from one to eight segregation granules, and in rare instances a few more than this. They contain on the average but three or four of these structures. In the monocytes the granules are always numerous, forty to sixty being a reasonable estimate of the number. The granules can be seen in fresh unaltered living cells without the use of dyes and are especially striking in dark field observations, but they are rendered clearest by supra-vital dyes which accentuate the segregation apparatus. Intermediate types between lymphocytes and monocytes are rare.

62. *Indirect Wallerian degeneration not found in all animals.* SUTHERLAND SIMPSON, Cornell University Medical College, Ithaca, New York.

The so-called retrograde degeneration, frequently found in some of the fibers in the central end of a divided nerve, is termed by Van Gehuchten 'indirect wallerian degeneration' and is accounted for by him as follows: When the motor root of one of the cranial nerves is divided by a sharp knife all the peripheral fibers, of course, undergo degeneration, but if the animal be kept alive for thirty or forty days after the operation some degenerated fibers may also be demonstrated by the Marchi method in the central end. If instead of making the lesion with a sharp knife he tore out the nerve, i.e., injured it as much as possible, then Van Gehuchten found that all the fibers in the central as well as the peripheral end underwent degeneration. When a nerve fiber is divided its cell of origin reacts and undergoes some degree of chromatolysis which is at its maximum in ten to fifteen days. If the lesion is made with a sharp knife the reaction is less marked, the cell recovers, never having ceased to function as a trophic center for the fiber, but, on the other hand, when the damage is very severe, it does not recover and at a certain stage in its downward course it is no longer able to act as a nutritive center to the fiber and from that time the latter undergoes Wallerian

degeneration. On repeating this work on the rabbit—the animal which Van Gehuchten used—the author is able to corroborate, but fails to obtain similar results in other species.

63. *A new classification of the pigmented tumors based on the cytology of the pigment cells.* DAVID T. SMITH, Johns Hopkins Medical School.

A study of sections from over 300 pigmented tumors from all parts of the human body has shown that these growths may be classified according to whether the tumor cells have produced the pigment or have taken it in as a foreign body. I. True pigmented tumors, in which the pigment is produced by the cells: These pigment granules are always individual, discrete bodies, all of about the same size, shape, and color in a given cell. True pigment tumors arise from pigmented cells belonging to two types of tissue—epithelium and mesothelium. The first type of cell is found in the skin and in the retina. In tissue cultures of the chick these pigmented cells grow as a membrane. They give rise to ordinary benign pigmented moles and to melanotic carcinoma (malignant melanoma). The second type of cell is long and spindle-shaped, with branched processes resembling the chromatophore of the lower animals. It occurs in the choroid of the eye, in membranes of the brain, along the nerve trunks, and in the core of the hair bulb. In chick tissue cultures these cells grow like connective tissue. They give rise to a second type of benign pigmented mole and to malignant melanotic sarcoma. II. Pigmented tumors, in which the pigment is not native to the cell but is taken in as a foreign body, as may be seen in the xanthomata and also in any tumors into which hemorrhage has occurred. In these cells the individual particles of pigment are irregular in size and shape and generally much larger than in the true pigmented tumors, in some instances almost filling the cell. It is evident that cells may sometimes be found in tumors of group I which contain foreign pigment having an origin similar to that found in cells of group II, but cells containing native pigment never occur in the tumors of group II.

64. *The effect of intraperitoneal injection of fresh anterior lobe substance in hypophysectomized tadpoles.* PHILIP E. SMITH and IRENE P. SMITH, Department of Anatomy, University of California.

As is well known, the early ablation of the buccal component of the hypophysis in the tadpole results in a well marked alteration in the pigmentary and endocrine systems, and in a slowing of the growth rate. The feeding of the fresh anterior lobe to these specimens brings about a nearly normal rate of growth, but does not bring to normality the altered pigmentary system, the specimens remaining typically albinous, nor do the internal secretory glands respond to this dietary regime, the thyroid failing to develop normally, the parathyroids being diminished in size, and the adrenal cortex diminished and atypically placed. These specimens do not metamorphose. Evans and Long have shown that the intraperitoneal injection of fresh anterior lobe substance in the normal rat produced a growth rate greatly in excess of the normal. Following their lead, we have tried the intraperitoneal injection of this substance in the hypophysectomized tadpole. Such injections bring about a striking change in the larvae so treated. The pigmentary and endocrine systems as well as the growth rate respond to this treatment. The animals become as dark as the normal but exhibit a slightly

more reddish tone. The adrenal cortex and thyroid do not exhibit the great reduction in size which they characteristically display in the typical albino. These animals reach a size much in excess of the normal. They completely metamorphose. From this it would appear that the successful treatment of individuals suffering from hypophyseal deficiency must be affected by the injection of the active principle of this gland and not by feeding, the treatment now resorted to and which has proven almost uniformly unsuccessful.

65. *The basic anatomical problem presented by achondroplasia and other unusual structural complexes.* CHARLES R. STOCKARD, Cornell University Medical College.

Achondroplasia and certain other peculiar structural conditions occurring in man and higher vertebrates are known in particular cases to be inherited through the male parent. This fact renders it probable that achondroplastic individuals arise fundamentally from new or strange chromosomal complexes. Several investigators have recently been inclined to interpret such conditions as due to some unusual activity on the part of glands of internal secretion. This interpretation is, in a sense, very probably true but a fuller consideration is necessary to determine the actual basic cause of the conditions.

Among lower mammals hybridization seems particularly disposed to give rise to such forms and in man this process may also play a part. The specimens in point are most common among chickens and dogs the breeds of which are often derived from more than a single wild species. In short, achondroplasia and similar conditions must be thought of as fundamentally genetic in origin. A mutation occurs and the resulting individuals are in no sense deformed specimens of the common norm.

In the hybrid and mutant individual a unique chromosomal organization was established at fertilization and following development each cell of the body is a new thing containing this strange chromosomal complex. Two propositions now present themselves; they are not antagonistic, and either one or both may be looked upon as explaining the new structural product. As the first possibility, the strange cellular complex of the developing animal may respond in a novel and definite way to the influence of usual or ordinary internal secretions, or in the second place, new and peculiar kinds of secretions may result from the mutation and the presence of these strange substances during development may cause deviations from the racial type, or finally, a combination of these two possibilities might readily obtain. A new breed thus occurs which is capable of perpetuating its characters and is a definite morphological entity or type. These conditions are considered in some detail.

66. *Some notes on the migration of neural crest cells in Rana palustris.* L. S. STONE, Yale University, School of Medicine.

This is a preliminary report of work in progress on normal and operated anuran embryos. Considerable similarity has been found in the wandering of neural crest cells of *Rana palustris* and *Amblystoma punctatum*. However, in *Rana palustris* the crest cells are well defined before the closure of the neural folds and operations involving the removal of these cells must be confined to this period, for at the closure of the folds the crest cells have already begun a rapid ventral

migration. The crest cells are not so easily followed in the earlier stages as in *Amblystoma*. They form the entire visceral skeleton. The removal of neural crest cells in the branchial region produces on that side an absence of the hyobranchial plate, ceratohyal, ceratobranchials and the corresponding half of the basibranchial. There is no contribution of entodermal mesenchyme to the visceral skeleton as in the case of the second basibranchial of *Amblystoma*. The anterior portions of the trabeculae are also formed from neural crest cells. A considerable portion of the connective tissue about the oral region as well as connective tissue in the regions of all the visceral bars is apparently derived from the neural crest.

67. *Significance of the fossa triangularis and cymba conchae.* G. L. STREETER, Department of Embryology, Carnegie Institution of Washington.

The fossa triangularis and the cymba conchae are part of the same structure and constitute an important articular surface of the cartilage of the auricle. As seen from a median view, they form in common a rounded eminence which is closely apposed to the side of the skull, being loosely attached by a fibrous ligament. This articular eminence is strengthened and better support is given to the auricle by the inferior crus of the anthelix, which projects into it as a sulcus from the median side and divides it into halves. The crus helcis takes part in the formation of the antero-lateral margin of the eminence.

68. *Hyperpigmentation and so-called blue skin.* R. M. STRONG, Loyola University School of Medicine.

In some cases of hyperpigmentation of human skin essentially no melanin pigment occurs in the epidermis. Numerous melanophores are present, however, in the dermis, especially in the papillae. Such cases, so far as known to the writer, exhibit so-called blue skin. No pathological conditions have been found associated with this pigmental abnormality, though intestinal disorders have preceded in the few cases where the history was available. In ordinary negro skin, there is a brownish tint which is lacking in these dermal pigmentation cases. Epidermal melanin occurs in the form of more or less distinctly separated brown granules. They are responsible for a brown tint. Similar granules occur in the dermal melanophores, but they are so clumped as to appear as a more or less definitely black mass. These melanophores give essentially no color to the light emerging from the skin. A dark gray results which lacks the familiar brownish tint and appears relatively blue. Black mixed with small amounts of white in the color wheel gives a similar affect. Carbon particles and other black foreign bodies inserted in the dermis also produced this appearance. If a real blue tint appears, the turbid medium hypothesis is the best explanation at present available for the blue component.

69. *The fate of the cells contained in the various quadrants of the primitive limb disc.* F. H. SWERT, Osborn Zoological Laboratory, Yale University.

Owing to the totipotence of the fore-limb rudiment of *Amblystoma punctatum*, it is impossible by simple extirpations to determine the portions of the limb formed by the various quadrants. Implantation of markers was attempted without result and the following method was devised. The mesoderm was stained

by heavily overstaining the animals *intra vitam* with Nile blue sulphate; the limb region was then covered with normal ectoderm, and anterior, dorsal, posterior, and ventral 'halves' of such limb rudiments were transplanted to corresponding positions in normal individuals. As the color remained visible until the division between the first two digits had appeared, it was possible to trace the cells to their ultimate positions in the limb. The anterior half contributes to the lateral side of the base, encroaching on the radial border and less on the extensor surface. The dorsal half contributes to the entire limb except the extreme anterolateral prominence of the shoulder. The posterior half contributes to the entire flexor surface and to the dorsum of the tip. The cells of the ventral half are distributed to the most anterolateral prominence of the shoulder and on the body wall ventral to the limb. Thus: 1) The anterodorsal quadrant forms the proximal part of the dorsal surface and radial border, also proximal parts of the shoulder. 2) The dorsoposterior quadrant forms the manus and distal part of the fore arm. 3) The posteroventral quadrant forms the proximal part of the flexor surface and contains cells which remain in the body wall. 4) The anteroventral quadrant contributes to the formation of the shoulder, but most of its cells do not enter the limb.

*70. Interrelation of thyroid and pituitary in producing metamorphosis.* W. W. SWINGLE, Osborn Zoological Laboratory, Yale University.

Transplantation of the pars anterior of adult frogs into immature larvae (*R. clamata*) with undifferentiated and physiologically inert thyroids, induces rapid growth and differentiation of the limbs, and eventually metamorphosis providing the graft survives. The thyroid apparatus of grafted animals reveals a marked development of the glands; they are larger than those of the controls and composed of large well-formed vesicles. Thyroids of control larvae remain comparatively undifferentiated with scanty colloid content. Pituitary grafts stimulate differentiation and physiological activity of the thyroids. The effect on body growth varies to such an extent interpretation is uncertain; in general results are negative.

Thyroids of large neotenuous tadpoles (extra season *R. clamata*, with hind legs 12 mm. long) transplanted into immature 33 mm. larvae without limb buds, induces precocious metamorphosis within thirty-five or forty days, yet such thyroids when left unmolested in the neotenuous individuals produce no metamorphic change in the same time interval. In fact such animals commonly pass an extra winter and spring in this stage. The experiment indicates that their thyroids are active and capable of inducing metamorphosis. Obviously, something hinders the release of the fully formed hormone into the blood stream of neotenuous larvae. The data suggests some defect of interrelation of the thyroid-pituitary mechanisms. The statement that thyroid transplants from neotenuous anurans into undifferentiated larvae induce metamorphosis only up to the stage reached by the larva from which the glands were taken is true only when the time interval is compared with that required by transplanted thyroids of newly metamorphosed frogs to transform similar individuals, other conditions being equal.

71. *Absence of monocytes in thoracic duct lymph.* GEORGE W. THORNE and H. M. EVANS, University of California.

The method of vitally staining leucocytes for the recognition of the monocytes as a distinct mononuclear cell class worked out in this laboratory by M. E. Simpson, has been applied in a study of the thoracic duct lymph. Inasmuch as the normal proportion of monocytes in the blood is low it would appear that the examination of lymph should be carried out in animals in which the proportion of monocytes has been experimentally increased; this may be secured by intravenous treatment of rabbits with some of the benzidine dyes. The monocytes constitute about 8 to 10 per cent of the leucocytes in normal rabbits. The animals were injected intravenously with aqueous solutions of 20 cc. of 1 per cent Niagara Blue at five day intervals. A monocytosis of 20 per cent or more has usually resulted. The vital benzidine dye injections are not always well tolerated. They may in fact cause a leukaemia due predominantly to toxicity to the myeloid cells (amphophils and basophils), and to a slighter extent to the lymphocytes. In such cases, monocytes may be even further increased in proportion and in absolute number. In one such instance there occurred a monocytosis so great that 75 per cent of the circulating leucocytes were monocytes.

It would seem probable from the study that monocytes and lymphocytes are not identical or closely related cell types, and furthermore, that if monocytes are produced in the lymphatic glands along with lymphocyte production, their rate of production is, however, insignificant. It is in fact more probable that the very low monocyte content of lymph is, like its erythrocyte content, due to the invasion of the lymph current by some of the blood elements.

72. *Metamorphosis of the cranium in orang.* T. WINGATE TODD, Western Reserve University.

Measurements as applied to the cranium are unsatisfactory mainly because the points utilized bear no relation to some common and relatively little variable feature. Underlying all individual, sexual, racial differences there is a common plan of formation, growth and metamorphosis which bears a definite relationship to the common plan in biological groups more comprehensive than the species. In order to discern the fundamental and relatively invariable points in the cranium, growth and metamorphosis must be separated. With Miss Margaret Russell as co-worker, I have chosen the orang as a suitable animal upon which to work out the postnatal metamorphosis of the cranium, first, because it is a giant Anthropoid and, secondly, because the cranial capacity in the adult orang is often no greater than in the animal presenting a full milk dentition. Miss Russell and I have turned our attention first to the cranial base and we find that there are areas of practically no growth (between infancy and adult age) interspersed among areas where great growth takes place, notwithstanding the fact that the cranial capacity is the same. There is therefore considerable movement of one unit of the cranium upon another during this period of life. In spite of these mutual and harmonic changes in relative position of cranial units we find a very steady and definitely predictable position for the external auditory meati and also for certain other points such as the basion and the foramina ovalia. We expect to apply this method to the remainder of the cranium and to other Anthropoids and Man after we have solved the metamorphosis of a cranium comparatively simple because of its meager postnatal increase in cranial capacity.

73. *The influence of feeding anterior lobe of hypophysis on growth and size of Amblystoma tigrinum.* (Lantern.) E. UHLENHUTH (introduced by E. V. Cowdry), Rockefeller Institute for Medical Research, New York.

Up to the present, there are very few experiments which support the view that the feeding of the anterior lobe of hypophysis has any influence on growth and size of the animals. Growth curves and photographs are shown in lantern slides; they demonstrate that anterior lobe, when fed to metamorphosed salamanders of the species *Amblystoma tigrinum*, causes an increase in the rate of growth and size, the animals becoming larger than those fed on other substances. If the animals are given anterior lobe of hypophysis, they grow at a higher rate than those which are fed earthworms. Moreover, they attain a size far in excess of that reached by worm-fed or liver-fed animals, and surpass the largest known animal of the species. At present, the largest hypophysis-fed animal is 19 per cent in excess of the largest liver-fed animal, 25 per cent larger than the largest known normal animal, and 37 per cent larger than the largest worm-fed animal. Liver-feeding causes a rate of growth higher than that resulting from an earthworm diet and equal to that produced by anterior-lobe-feeding. But the liver-fed animals stop growing at a size at which anterior lobe-fed specimens of the same age continue to grow vigorously. Liver-fed animals may grow larger than worm-fed animals and may exceed the size of the largest known animal of the species by as much as 4.7 per cent, but they do not reach the size of hypophysis-fed animals.

74. *The musculature of the stomach of the fetus and newborn.* ELWYN H. WELCH (introduced by R. E. Scammon), Department of Anatomy, University of Minnesota.

The arrangement of gastric musculature of older human fetuses and the newborn may be summarized as follows: 1. The external longitudinal layer is continuous with that of the esophagus and the duodenum. It completely invests the stomach but is very much thinned or even deficient at the mid-point of the dorsal and ventral surfaces. This coat is much heavier over the lesser curvature and in the regions of the cardia and of the pylorus. 2. The so-called middle coat of circular fibers is arranged in concentric rings from the duodenum to the cardia where they are slightly more numerous on the right and superior aspect. Over the fundus these fibers are incomplete and terminate by tucking-in to the circular fibers of the fundus which apparently have a distinctly separate origin. These latter fibers radiate from the center of the fundic dome and completely surround this portion of the stomach. 3. On the left side of the esophageal-cardiac junction, the region of the incisura cardiaca, the oblique or innermost layer bears the same relation as the circular fibers on the right side. This band is continuous above with the deep circular fibers of the esophagus and below with the circular fibers of the fundus. Its fibers course over the lateral wall in the direction of the pylorus ending by turning-in to and becoming a part of the overlying circular layer. At birth some of these fibers can be traced quite to the pylorus.

## DEMONSTRATIONS

1. *Radiographs showing the blood supply of the pelvic viscera.* H. S. BURR, School of Medicine, Yale University.
2. *Microscopic preparations showing the transformed bladder muscle.* EBEN J. CAREY, Marquette School of Medicine.
3. *Flagellated thyroid cells in the dog-fish.* E. V. COWDRY, Rockefeller Institute.
4. *Grafts of chick embryo analages in the allantois.* VERA DANCHAKOFF, Columbia University.
5. *Growth of the medullary tube grafted into allantois.* ANNA AGASSIZ and VERA DANCHAKOFF, Columbia University.
6. *Injection of the vascular system of the bone-marrow of the pigeon.* C. A. DOAN (introduced by Florence R. Sabin), Johns Hopkins Medical School.
7. *Demonstration of preparations illustrating a strain of epithelial cells in pure culture.* ALBERT FISCHER (introduced by E. V. Cowdry), Rockefeller Institute for Medical Research.
8. *The effect of the extirpation of the embryonic eye and ear on equilibration in Amblystoma punctatum. Demonstration of live specimens.* WALTER F. GREENE, (introduced by R. G. Harrison), Yale University.
9. *Injected pig embryos dissected and cleared to show the fate of the right dorsal aorta.* CHESTER H. HEUSER, Department of Embryology, Carnegie Institution.
10. *Preliminary results of rotation of portions of the embryonic spinal cord about its long axis.* DAVENPORT HOOKER, University of Pittsburgh School of Medicine.
11. *Slides showing mitochondria and secretion granules and vacuoles in hyperactive human thyroids.* J. ALBERT KEY, Harvard Medical School.
12. *Methylene-blue slides of nerves and nerve-terminations in the lung.* O. LARSELL, University of Oregon Medical School.
13. *Pantographic reproductions of radiographs showing the position of abdominal viscera in healthy young adults.* ROBT. O. MOODY, University of California.
14. *Lantern slide demonstration of congenital abnormality of the hands and right limb.* H. S. MURPHEY, Iowa State College.
15. *Dorsal and ventral implantations of the limb bud.* J. S. NICHOLAS, University of Pittsburgh School of Medicine.
16. *Demonstration of preparations showing the reaction of the iron-containing proteins of the cells of the nucleus hypoglossus following axone injury.* F. M. NICHOLSON (introduced by R. R. Bensley), University of Chicago.
17. *Drawings, cleared specimens, and x-ray pictures illustrating the ossification of the laryngeal cartilages.* G. J. NOBACK, Medical College of Virginia.
18. *Graphs and curves illustrating the prenatal growth of the several parts of the respiratory system in man.* G. J. NOBACK, Medical College of Virginia.

The dissections and measurements were made on a series of specimens, both fresh and preserved, in the collection of the Institute of Anatomy, University of Minnesota. The graphs and curves were made by plotting the measurements against the total body length. The curves approximate straight lines in all but a few of the 46 measurements plotted.



19. *Demonstration of sound transmission phenomena.* A. G. POHLMAN, St. Louis, University.

These demonstrations require the setting up of apparatus—time and place will be announced. 1. The manner in which the auricle overcomes the damping effect of the external auditory canal (air transmission). 2. The manner in which the stapedial foot-plate enhances a discharge of energy into the perilymph (solid to liquid transmission). 3. The diaphragm-rod theory of sound transmission (air to solid to liquid transmission). 4. The capacity of bone, tendon, elastic and muscle for sound transmission. 5. The piezo-electric reaction of the Rochelle salt transmitter to air, liquid, and solid transmitted sound. The writer expresses his obligation to Dr. F. B. Jewett of the Western Electric Research laboratory, N. Y., for the loan of this transmitter.

20. *A preliminary report of a study of the human femur.* J. A. POLCZAK and EDWIN J. SIMONS (introduced by G. J. Noback), University of Minnesota.

A series of 248 human femurs have been measured and indexed to date. They consist of 48 fresh (paired) specimens and 200 from the osteological collection of the Institute of Anatomy. They are from peoples of various nationalities but the majority are of Scandinavian origin. The technic employed is the same as that employed by Parsons and by Holtby. The left femur is greater than the right in the following measurements and indices: Maximal Length, Oblique Length, Least Transverse Diameter, Bi-condylar Width, Diameter of the Head; Head Length Index, Platymetric Index, and Pilasteric Index. The above is true of the majority of the left femurs; however, when either femur is found to predominate in any one of its measurements or indices it generally predominates in all the others. The series is not yet completed and further work is being done on sexing.

21. *Demonstration of developing blood-cells.* FLORENCE R. SABIN, Department of Anatomy, Johns Hopkins Medical School.

22. *Field graphs and curves illustrating the growth of the human eyeball and optic nerve.* R. E. SCAMMON and E. L. ARMSTRONG, University of Minnesota.

23. *Graphs and curves illustrating the transition from the fetal to the postnatal growth phases in man as shown by the projection of empirical formulae of fetal growth and by data on the growth increments of premature infants.* R. E. SCAMMON, University of Minnesota.

24. *Graphs and curves illustrating the prenatal growth of the human mandible.* R. E. SCAMMON and S. J. Z. GANZ, University of Minnesota.

25. *An orthoscopic apparatus for graphic reconstruction.* R. E. SCAMMON, University of Minnesota.

26. *Graphs and reconstructions illustrating the topography of the abdominal viscera in later embryonic and in fetal life.* A. L. CAMERON, University of Minnesota.

27. *Graphs and curves of the postnatal growth of the human brain and spinal cord.* R. E. SCAMMON and H. L. DUNN, University of Minnesota.

28. *Graphs, curves and empirical formulae of surface area in man and animals.* H. L. DUNN (introduced by R. E. Scammon), University of Minnesota.

29. *Preparations to show the effects of inanition upon the mitochondria in the gastrointestinal mucosa and in the pancreas of the albino rat.* SHIRLEY P. MILLER (introduced by C. M. Jackson), University of Minnesota.

30. *Graphs illustrating the postnatal growth in weight of the various organs and parts of the guinea pig.* A. N. BESSESEN, JR. and H. A. CARLSON (introduced by C. M. Jackson), University of Minnesota.
31. *Gross sections of an infant prepared and mounted by the glycerine-gelatine picture-frame method.* CARL CASKEY SPEIDEL, University of Virginia; and ROY M. HOOVER, New York Orthopaedic Hospital.
32. *Demonstration of human skin showing dermal pigmentation only, and exhibiting the phenomenon of so-called blue skin.* R. M. STRONG, Loyola University School of Medicine.
33. *Cleared embryos illustrating the order, time and rate of ossification in the rat skeleton.* R. M. STRONG, Loyola University School of Medicine.
34. *Radiograph of a syndactylous hand, with an interpretative diagram.* W. E. SULLIVAN, University of Wisconsin.
35. *Dissection of a cod. To illustrate the function of articular discs.* W. E. SULLIVAN, University of Wisconsin.
36. *The effect of anterior lobe-feeding on the size of *Ambystoma tigrinum*.* E. UHLENHUTH (introduced by E. V. Cowdry), Rockefeller Institute for Medical Research.

Live specimens of *Ambystoma tigrinum* are shown to demonstrate the effect of excessive feeding of the anterior lobe of the hypophysis on the size of adult salamanders. The hypophysis-fed salamanders exceed all the control animals in size.

37. *Initial stages in the function of the suprarenal medulla in pig embryos.* M. F. WEYMANN (introduced by C. H. Danforth), Department of Anatomy, Washington University School of Medicine.

Several investigators have maintained that the Henle reaction of cells in the suprarenal medulla is due to the presence of epinephrin in the cytoplasm. Test-tube experiments have tended to confirm this idea, since it is found that the precipitate formed when pure epinephrin is added to Müller's fluid is soluble in the same reagents as are the granules found within the cells showing the Henle reaction. These facts are believed to justify the assumption that the intensity of Henle's reaction furnishes an index of the degree of function in the suprarenal medulla. The specimens exhibited are suprarenal glands from pig embryos in different stages of development, fixed in Müller's fluid, hardened in alcohol, sectioned, and stained with neutral haematoxylin and eosin. This series of specimens indicates that: 1) the assumption of a secretory function by the medullary cells is a gradual process which is not complete until about the 142 mm. stage, after which the reaction appears to be fairly constant; 2) with the beginning of function the cells change in character from a more or less embryonic form with a large loose-meshed nucleus to a more specialized form with a smaller more deeply staining nucleus; 3) cells begin to show the reaction even while outside the cortex and at a considerable distance from their ultimate site; 4) all cells do not begin to function at the same time; 5) a few of them already show signs of function at the 45 mm. stage.

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- SMITH, GEORGE MILTON, A.B., M.D., Attending Surgeon, *Waterbury Hospital, 111 Buckingham Street, Waterbury, Conn.*
- SMITH, GRAFTON ELLIOT, M.A., M.D., F.R.S., Professor of Anatomy, *University College, Gower St., London, W. C. 1, England.*
- SMITH, H. P., A.B., *Hooper Research Lab., 1332 Sixth Ave., San Francisco, Calif.*
- SMITH, PHILIP EDWARD, M.S., Ph.D., Assistant Professor of Anatomy, *University of California, 1513 Scenic Avenue, Berkeley, Calif.*
- SMITH, WILBUR CLELAND, M.D., Surgeon, *Americus, Ga.*

- SNOW, PERRY G., A.B., M.D., Dean and Professor of Anatomy, *University of Utah Medical School, Salt Lake City, Utah.*
- SPAULDING, M. H., A.M., Assistant Professor of Zoology, *University of Montana, Bozeman, Montana.*
- SPEIDEL, CARL C., Ph.D., Adjunct Professor of Anatomy, *University of Virginia, University, Va.*
- STARK, MARY, Ph.D., Professor of Histology and Embryology, *New York Homeopathic College, New York City.*
- STEVENSON, PAUL H., M.D., Assistant in Anatomy, *Peking Union Medical College, Peking, China.*
- STEWART, CHESTER A., A.M., Ph.D., Fellow in Pediatrics, *Room 121, Millard Hall, University of Minnesota, Minneapolis, Minn.*
- STEWART, FRED WALDORF, A.B., Ph.D., Instructor in Neurology, *Cornell University Medical College, Ithaca, N. Y.*
- STILES, HENRY WILSON, M.D., Professor of Anatomy, College of Medicine, *Syracuse University, 309 Orange Street, Syracuse, N. Y.*
- STOCKARD, CHARLES RUPERT, M.S., Ph.D., Sc.D., (Secretary-Treasurer '14-'21), Professor of Anatomy, *Cornell University Medical College, New York City.*
- STONE, LEON STANSFIELD, Ph.B., Assistant in Anatomy, *Medical College, Yale University, New Haven, Conn.*
- STONE, ROBERT S., B.A., Assistant in Anatomy, *University of Toronto, 40 Wilcox St., Toronto, Ontario, Canada.*
- STOPFORD, JOHN SEBASTIAN B., M.D., Professor of Anatomy, *University of Manchester, Manchester, England.*
- STOTSENBURG, JAMES M., M.D., Instructor in Anatomy, *The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.*
- STREETER, GEORGE L., A.M., M.D., (Ex. Com. '18-'20), Director Department of Embryology, *Carnegie Institution of Washington, Johns Hopkins Medical School, Baltimore, Md.*
- STROMSTEN, FRANK ALBERT, D.Sc., Associate Professor of Animal Biology, *State University of Iowa, 321 East College St., Iowa City, Iowa.*
- STRONG, OLIVER S., A.M., Ph.D., Associate Professor of Neurology, *Columbia University, 437 West 59th Street, New York City.*
- STRONG, REUBEN MYRON, A.M., Ph.D. (Ex. Com. '16-'19), Professor of Anatomy, *Loyola University School of Medicine, 706 South Lincoln Street, Chicago, Ill.*
- SULLIVAN, WALTER EDWARD, A.M., Ph.D., Assistant Professor of Anatomy, *University of Wisconsin, Science Hall, Madison, Wis.*
- SUNDWALL, JOHN, Ph.D., M.D., Professor of Hygiene, *University of Minnesota, Minneapolis, Minn.*
- SUTTON, ALAN CALLENDER, A.B., M.D., Johns Hopkins Medical School, *3212 Abell Avenue, Baltimore, Md.*
- SWETT, FRANCIS HUNTINGTON, A.M., Assistant, *Osborn Zoological Laboratory, Yale University, New Haven, Conn.*
- SWIFT, CHARLES H., M.D., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Chicago, Chicago, Ill.*
- SWINGLE, W. W., Ph.D., Assistant Professor of Biology, *Sheffield Scientific School, Yale University, New Haven, Conn.*

- SYKES, GEO. F., Ph.B., M.A., Professor of Zoology and Physiology, *Oregon State Agricultural College, Corvallis, Oregon.*
- TAKENOUCHI, MATSUZIRO, M.D., Assistant Professor of Bacteriology and Hygiene, *Medical College, Imperial University of Tokio, Tokio, Japan.*
- TERRY, ROBERT JAMES, A.B., M.D. (Ex. Com. '08-'12, '21-), Professor of Anatomy, *Washington University Medical School, St. Louis, Mo.*
- THOMSON, ARTHUR, M.A., M.B., LL.D., F.R.C.S., Professor of Anatomy, *University of Oxford, Department of Human-Anatomy, Oxford, England.*
- THORKELSON, JACOB, M.D., *Daly Bank Bldg., Anaconda, Montana.*
- THRO, WILLIAM C., A.M., M.D., Professor of Clinical Pathology, *Cornell University Medical College, 28th Street and 1st Avenue, New York City.*
- THÜRINGER, JOSEPH M., M.D., Professor of Histology and Embryology, *University of Oklahoma, Norman, Okla.*
- THING, FREDERICK WILBUR, Ph.D., Professor of Anatomy, *Tufts Medical College, 416 Huntington Ave., Boston, Mass.*
- TILNEY, FREDERICK, M.D., Ph.D., Professor of Neurology, *Columbia University, 22 East 63rd Street, New York City.*
- TODD, T. WINGATE, M.B., Ch.B. (Manc.), F.R.C.S. (Eng.), (Vice-Pres. '20-'21), Professor of Anatomy, Medical Department, *Western Reserve University, 1353 East 9th Street, Cleveland, Ohio.*
- TRACY, HENRY C., A.M., Ph.D., Professor of Anatomy, *University of Kansas, Lawrence, Kansas.*
- TUPPER, PAUL YOER, M.D., Clinical Professor of Surgery, *Washington University Medical School, Wall Building, St. Louis, Mo.*
- TURNER, CLARENCE L., M.A., Ph.D., Professor of Zoology, *Beloit College, Beloit, Wisconsin.*
- UHLENHUTH, EDUARD, Ph.D., Associate in Pathology, *Rockefeller Institute for Medical Research, 66th Street and Avenue A, New York City.*
- VANCE, HARRY WELLINGTON, A.B., Medical Student, *Johns Hopkins Medical School, Baltimore, Md.*
- VAN DER HORST, C. J., Ph.D., *Zoologisch Laboratorium, Pl. Muidergracht 34, Amsterdam, Holland.*
- VAN DER STRICHT, OMER, M.D., Professor of Histology and Embryology, *University of Ghent, 11 Marché au lin, Ghent, Belgium.*
- WAITE, FREDERICK CLAYTON, A.M., Ph.D., Professor of Histology and Embryology, *Western Reserve University School of Medicine, 1353 East 9th Street, Cleveland, Ohio.*
- WALLIN, IVAN E., M.A., D.Sc., Professor of Anatomy, *University of Colorado, College of Medicine, Boulder, Colo.*
- WALMSLEY, THOMAS, M.D., Professor of Anatomy, *Queens University of Belfast, Belfast, Ireland.*
- WARREN, JOHN, A.B., M.D., Associate Professor of Anatomy, *Harvard Medical School, Boston, Mass.*
- WATERSTON, DAVID, M.A., M.D., F.R.C.S. Ed., Butte Professor of Anatomy, *University of St. Andrews, St. Andrews, Fife, Scotland.*

- WATKINS, RICHARD WATKIN, B.S., Instructor in Anatomy, *Department of Anatomy, University of Chicago, Chicago, Ill.*
- WATSON, DAVID MEREDITH SEARS, M.Sc., Lecturer in Vertebrate Paleontology, *University College, Gower St., London, W. C. 1, England.*
- WATSON, ERNEST M., A.M., M.D., Instructor in Applied Anatomy, *University of Buffalo, 494 Franklin St., Buffalo, N. Y.*
- WATT, JAMES CRAWFORD, M.A., M.D., Assistant Professor of Anatomy, *University of Toronto, 20 Hawthorne Avenue, Toronto, Canada.*
- WEED, LEWIS HILL, A.M., M.D., (Ex. Com. '20-'21, Sec.-Treas. '22-), Professor of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- WEGEFORTH, PAUL, A.B., M.D., Captain M. C., U. S. A., *Coronado, Calif.*
- WEIDENREICH, FRANZ, M.D., a.o. Professor and Prosector of Anatomy, *formerly 19 Vogesen Street, Strassburg, i Els. France.*
- WEST, RANDOLPH, A.M., M.D., *69 East 93rd St., New York, N. Y.*
- WHITE, HARRY OSCAR, M.D., *University Club, Los Angeles, Calif.*
- WHITESIDE, BEATRICE, Ph.D., Instructor in Anatomy, *Washington University Medical School, St. Louis, Mo.*
- WHITNALL, S. E., M.A., M.D., B.Ch., Professor of Anatomy, *McGill University, Montreal, Canada.*
- WITTENBORG, A. H., M.D., Professor of Anatomy, *College of Medicine, University of Tennessee, Memphis, Tenn.*
- WILDER, HARRIS HAWTHORNE, Ph.D., Professor of Zoölogy, *Smith College, Northampton, Mass.*
- WILHELMJ, CHARLES M., A.B., Teaching Fellow in Anatomy, *St. Louis University Medical School, 1402 South Grand Ave., St. Louis, Mo.*
- WILLIAMS, STEPHEN RIGGS, A.M., Ph.D., Professor of Zoölogy and Geology, *Miami University, 300 East Church Street, Oxford, Ohio.*
- WILLARD, WILLIAM A., A.M., Ph.D., Professor of Anatomy, *University of Nebraska, College of Medicine, 42d Street and Dewey Avenue, Omaha, Neb.*
- WILSON, J. GORDEN, M.A., M.B., C.M. (Edin.), Professor of Otology, *Northwestern University Medical School, 2481 S. Dearborn Street, Chicago, Ill.*
- WILSON, JAMES THOMAS, M.B., F.R.S., Professor of Anatomy, *University of Cambridge, New Museums, Cambridge, England.*
- WILSON, LOUIS BLANCHARD, M.D., Director of Pathology Division, *Mayo Clinic and Mayo Foundation, Professor of Pathology in the University of Minnesota, Mayo Clinic, 850 W. College Street, Rochester, Minn.*
- WISLOCKI, GEORGE B., A.B., M.D., Associate in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- WITHERSPOON, THOMAS CASEY, M.D., *Murray Hospital, Butte, Mont.*
- WOODMAN, A. S., M.D., Assistant Professor of Histology and Embryology, *Boston University School of Medicine, Boston, Mass.*
- WOOLLARD, HERBERT T., M.D., Demonstrator of Anatomy, *University College, Gower St., London, W. C. 1, England.*
- WORCESTER, JOHN LOCKE, M.D., Professor of Anatomy, *University of Washington, 5211-21st Avenue, N.E., Seattle, Wash.*

## PROCEEDINGS OF THE AMERICAN SOCIETY OF ZOOLOGISTS

The American Society of Zoölogists held its Nineteenth Annual meeting at the University of Toronto in conjunction with Section F of the American Association, and in association with other biological societies, December 28, 29, and 30, 1921.

Officers for the year were:

*President:* CHARLES A. KOFOID.

*Vice-President:* AARON L. TREADWELL.

*Secretary-Treasurer:* W. C. ALLEE.

*Executive Secretary, Genetics Section:* L. J. COLE.

*Local Committee:* A. G. HUNTSMAN, *Chairman*, J. M. D. OLMSTED, LUCY CLEMENS.

*Executive Committee:* H. V. WILSON, M. M. METCALF, GEORGE LEFEVRE, C. M. CHILD, GILMAN A. DREW.

*Membership on the Council of the A. A. A. S.:* C. C. NUTTING and W. C. ALLEE.

*Representatives of the Society in the Division of Biology and Agriculture of the  
National Research Council*

	<i>Term expires</i>
F. R. LILLIE.....	1923
WILLIAM PATTEN.....	1924
G. H. PARKER.....	1922

### EDITORIAL BOARD OF THE JOURNAL OF MORPHOLOGY

*Managing Editor* (Term expires 1926).....C. E. McCLUNG

#### *Associate Editors*

To serve until 1922.....	{ GARY N. CALKINS J. S. KINGSLEY WILLIAM PATTEN
To serve until 1923.....	{ E. G. CONKLIN M. F. GUYER W. M. WHEELER
To serve until 1924.....	{ C. A. KOFOID F. R. LILLIE J. T. PATTERSON

## BUSINESS MEETING

At the annual business meeting the appended report of the Treasurer and the Auditing Committee was accepted and ordered to be placed on file.

*Expenditures in 1921*

To The Wistar Institute for subscriptions:	
January 6.....	\$68.00
February 25 .....	4.50
June 6.....	1,971.50
July 4.....	11.00
August 14.....	48.00
October 25.....	28.00
November 3.....	.50
December 19.....	116.00
	<hr/>
	\$2,247.50
Expenses of office of Secretary-Treasurer:	
Printing nomination blanks.....	\$13.50
To The Wistar Institute for programs, envelopes, etc.....	63.10
Telephone and telegrams.....	10.06
Stenography, typewriting, etc.....	57.00
Stationery.....	1.20
Petty cash.....	18.52
Fare of Secretary-Treasurer to Toronto.....	26.65
Miscellaneous:	
Expenses V. E. Shelford to Des Moines.....	60.50
Overpayment of dues.....	2.50
Foreign exchange.....	3.95
Correction (error of bank clerk).....	10.00
	<hr/>
	\$266.98
	<hr/>
	2,247.50
	<hr/>
Total expenditures.....	\$2,514.48

*Receipts in 1921*

Balance on hand at last report.....	\$960.00
\$5 \$5.00 dues.....	425.00
233 \$7.00 dues.....	1,631.00
25 \$11.50 dues.....	287.50
Irregular dues.....	73.34
Interest on savings.....	45.39
	<hr/>
Money held or received in 1921.....	\$3,422.57



Cash on hand, December 26, 1921:	
Savings account.....	\$906.96
Checking account.....	1.13
	<hr/>
	\$908.09
Total expenditures in 1921.....	\$2,514.48
Plus cash on hand.....	908.09
	<hr/>
	\$3,422.57

We have examined the accounts of the treasurer and found them correct.

A. S. PEARZE,  
CHARLES ZELENY,  
*Auditing committee.*

*Probable balance January 1, 1922*

Balance, December 26, 1921.....	\$908.09
Approximate interest on savings.....	12.50
Final dividend on trust fund liquidation.....	8.06
	<hr/>
	\$928.65
Bills payable January 1:	
The Wistar Institute for subscriptions.....	80.00
Expenses of Secretary's office.....	40.00
	<hr/>
Probable balance January 1, 1922.....	\$808.65
Balance, January 1, 1921.....	\$89.30
	<hr/>
Probable loss for the year.....	\$81.71

In addition, there are 31 members or ex-members in arrears for a total of 43 years, aggregating dues amounting to \$293.50.

*Election of members*

The appended list of zoölogists were duly nominated for membership in the Society, as having shown evidence of continued contribution to zoölogical research, and were elected.

ADOLPH, EDWARD FREDERICK, A.B., Ph.D. (Harvard), Instructor in General Physiology, University of Pittsburgh, *Biology Hall, University of Pittsburgh, Pittsburgh, Pa.*

ALEXANDER, CHARLES PAUL, B.S., Ph.D. (Cornell), Systematic Entomologist for the State Natural History Survey of Illinois, *Natural History Bldg., 316 A, Urbana, Illinois.*

ALLEN, WILLIAM RAY, A.B., A.M., Ph.D. (Indiana), Associate Professor of Zoölogy, University of Akron, *University of Akron, Akron, Ohio.*

BAKER, HORACE BARRINGTON, B.S., Ph.D. (Michigan), Instructor of Zoölogy, University of Pennsylvania, *Zoölogical Laboratory, University of Pennsylvania, Philadelphia, Pa.*

- BLANCHARD, FRANK N., A.B. (Tufts), Ph.D. (Michigan), Instructor in Zoölogy, University of Michigan, *University of Michigan, Ann Arbor, Mich.*
- BODINE, JOSEPH HALL, A.B., Ph.D. (Pennsylvania), Instructor in Zoölogy, University of Pennsylvania, *Zoölogical Laboratory, University of Pennsylvania, Philadelphia, Pa.*
- BOWEN, ROBERT HALL, A.B. (Colby), A.M., Ph.D. (Columbia), Instructor in Zoölogy, Columbia University, *Department of Zoölogy, Columbia University, New York City.*
- CAMERON, ALFRED ERNEST, M.A., B.Sc. (Aberdeen), M.Sc. (Manchester), D.Sc. (Aberdeen), Professor of Zoölogy, University of Saskatchewan, *616, Lansdowne Avenue, Saskatoon, Sask.*
- COLE, WILLIAM H(ARDER), A.B., A.M., Ph.D. (Harvard), Professor of Biology, Lake Forest College, *Lake Forest, Illinois.*
- COLLETT, MARY ELIZABETH, A.B. (Wellesley), A.M., Ph.D. (Pennsylvania), Instructor in Physiology, Dept. of Medicine, University of Buffalo, *University of Buffalo (Medical), 24 High Street, Buffalo, N. Y.*
- COWLES, RHEINART P., A.B. (Stanford), Ph.D. (Hopkins), Associate Professor of Zoölogy, *Johns Hopkins University, Baltimore, Md.*
- DAWSON, ALDEN BENJAMIN, A.B. (Acadia), Ph.D. (Harvard), Assistant Professor Microscopical Anatomy, Loyola University School of Medicine, *706 S. Lincoln St., Chicago, Illinois.*
- ELLINGER, TAGE U.H., M.S. (Copenhagen), Statsstipendiat (Danish Government), Fellow of American-Scandinavian Foundation, *1105 Busey Ave., Urbana, Illinois.*
- HOPKINS, HOYT STILSON, A.B., A.M. (Oberlin), Ph.D. (Hopkins), Assistant Professor of Physiology, *Baylor Medical College, Dallas, Texas.*
- HUBBS, CARL L., A.B., A.M. (Stanford), Curator and Instructor, *Museum of Zoölogy, University of Michigan, Ann Arbor, Michigan.*
- HUNTER, GEORGE WILLIAM, A.B., A.M. (Williams), Ph.D. (New York University), Professor of Biology, Knox College, *Galesburg, Illinois.*
- LANCEFIELD, DONALD E., A.B. (Reed), M.A., Ph.D. (Columbia), Assistant Professor of Zoölogy, *University of Oregon, Eugene, Oregon.*
- MACARTHUR, JAMES WOOD, A.B. (Oberlin), M.A. (Wabash), Ph.D. (Chicago), Lecturer in Genetics and Experimental Biology, University of Toronto, *Department of Biology, University of Toronto, Toronto, Canada.*
- McEWEN, ROBERT STANLEY, A.B., A.M. (Western Reserve), Ph.D. (Columbia), Assistant Professor of Zoölogy, Oberlin College, *Spear Laboratory, Oberlin College, Oberlin, Ohio.*
- OKKELBERG, PETER, A.B., A.M. (Minnesota), Ph.D. (Michigan), Assistant Professor of Zoölogy, University of Michigan, *1116 Ferdon Road, Ann Arbor, Mich.*
- PARMENTER, CHARLES LEROY, A.B., M.A. (Southern California), Ph.D. (Pennsylvania), Instructor of Zoölogy, University of Pennsylvania, *Zoölogical Laboratory, University of Pennsylvania, Philadelphia, Pa.*
- PINNEY, MARY EDITH, A.B., A.M. (Kansas State), Ph.D. (Bryn Mawr), Professor of Biology, Lake Erie College, *Painesville, Ohio.*
- REAGAN, FRANKLIN PEARSE, A.B. (Indiana), Ph.D. (Princeton), Assistant Professor of Zoölogy, University of California, Chairman of the Department, *1921-2, 219 East Hall, University of California, Berkeley, California.*

- RHODES, ROBERT C(LINTON), A.B. (Henderson Brown), A.B., A.M., (Vanderbilt), Ph.D. (California), Professor of Biology, *Emory University, Ga.*
- SCHRADER, FRANZ, B.S., Ph.D. (Columbia), Associate in Biology, *Bryn Mawr College, Bryn Mawr, Pa.*
- STEINER, GOTTHOLD, Ph.D. (Berne), Privatdocent in Zoölogy, University of Berne, Seesel Research Fellow, Yale University, 1921-22, *Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*
- STUNKARD, HORACE W., B.S. (Coe), A.M., Ph.D. (Illinois), Assistant Professor of Zoölogy, *New York University, University Heights, New York City.*
- WEED, LEWIS HILL, A.B., A.M. (Yale), M.D. (Hopkins), Professor of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- WOODWARD, ALVALYN E., A.B., M.S. (Rochester), Ph.D. (Michigan), Demonstrator in Biology, *Amherst College, Amherst, Mass.*
- YOUNG, BENJAMIN P., B.S. (Kansas), Ph.D. (Cornell), Assistant Professor of Zoölogy, *Cornell University, 209 Eddy St., Ithaca, N. Y.*
- YŪASA, HACHIRO, B.S. (K.S.A.C.), M.S., Ph.D. (Illinois), Research Entomologist, Illinois State Natural History Survey, *Natural History Bldg., University of Illinois, Urbana, Ill.*

WILLIAM BATESON, Director of the John Innis Horticultural Institution, Morton Park, Surrey, England, and joint guest of The American Society of Zoölogists and The American Association for the Advancement of Science, at Toronto, was elected an honorary fellow of the Society.

The Secretary reported that five members were two years or more in arrears for dues, and these were accordingly no longer members of the Society, according to Art. II, Sec. 3, of the Constitution. Of six members dropped in 1920, three had been reinstated on payment of dues to date. The total membership at present is 357.

Attention of the Society was called to the agreement with the Wistar Institute, which is reprinted below:

### *The Wistar Journals*

It has recently become apparent that some members do not understand the nature of the agreement between the American Society of Zoologists and The Wistar Institute regarding the Wistar journals. For this reason a statement written by Caswell Grave early in 1916, is re-printed here. Professor Grave was Secretary-Treasurer for the Society at the time and was active in the negotiations between the Society and The Wistar Institute which led to the present arrangement.

## WHY THE PROPOSITION WAS MADE AND ACCEPTED

From The Wistar Institute standpoint the proposition was made for these reasons:—

- a) To place the Journals in the hands of those who are most active in the promotion of Zoölogical research.
- b) To unite in a "Triple Alliance" the two Societies and an endowed research institute for organized effort in all that pertains to the progress of Zoological science.

A special fund has been raised to accomplish these purposes with the least possible tax upon the individual members of the Society, and to aid in the prompt publication and extensive distribution of the rapidly increasing number of papers accepted for publication.

From the standpoint of the Society it seemed a duty of the Society to come to the aid of American Zoölogical Journals at this time and assist in placing them upon a basis such that they may serve the purposes for which they were established, and provide an adequate means for publishing and distributing the work of American biologists.

A proposition, whereby members of the Society might for \$6.50 receive Journals representing a value of \$42.00, also seemed attractive even to a Zoologist with small salary, and one not to be lightly turned down.

## THE AGREEMENT

Between the American Society of Zoölogists and The Wistar Institute there is no formal agreement in this matter, but, in making the proposition, The Wistar Institute stated in its printed circular and in the copy presented at the meeting that this offer is to members only—and that the end aimed at by the Institute will be defeated if the Journals intended only for the reading tables of members are allowed to reach University or College libraries, thus causing the loss of subscriptions made by Institutions at the regular rate; and, in accepting the proposition, the Society morally bound its members not to allow journals, secured by them at the low Society Rate, to be substituted for journals subscribed for by Institutions at *regular rates*.

It is understood, however, that previous subscriptions by members to these journals at regular rates need not be kept up.

In reply to an inquiry whether the Journal proposition could be made to the Society provided 50% or 75% of the membership were guaranteed by the Society to accept, the director of the Wistar Institute stated that the low Society rate on the Journals could be made only in case a sum is paid by the Society equivalent to \$6.50 for each regular member and provided the name of every such member is added to the mailing lists of the journals.

There have been no changes in the agreement with The Wistar Institute since that time, and so far as the present Secretary-Treasurer can learn, the arrangement has worked well. It is certainly true that members of The American Society of Zoölogists get more journals for the money invested than any other Society which supports a publication program, excepting only the Anatomists, who have a similar agreement with The Wistar Institute. It would cost approximately half our annual dues to print and circulate the Announcement, Abstracts, Proceedings and Membership List which under the present arrangement cost about twenty cents per member per year.

The Secretary was instructed to prepare a formal notification blank to be used in informing new members of their election,

of the privileges of members, and their responsibilities with regard to the Society's journal arrangement.

Proposed amendments made in accordance with Article VI of the Constitution, allowing for the separation of the office of Secretary-Treasurer, were adopted. The By-Laws are also amended to the same end. The Constitution and By-Laws, as amended, will be found on pages 134-7.

### *Report of the Nominating Committee*

The Nominating Committee (see Art. III, Sec. 6), composed of M. F. Guyer, S. J. Holmes and J. H. Gerould, report the following nominations:

*President*—H. H. WILDER.

*Vice-President*—B. M. ALLEN.

*Secretary*—W. C. ALLEE.

*Treasurer*—D. H. TENNENT.

*Member Executive Committee*—C. A. KOFOID.

*Member National Research Council*—H. S. JENNINGS.

*Three Associate Editors of the Journal of Morphology*—L. L. WOODRUFF, G. A. DREW and H. V. NEAL.

*Membership in Council, A. A. A. S.*—CHARLES ZELENY and H. E. CRAMPTON.

Nominations from the floor were called for; none were presented and the proposed ticket was elected.

In accordance with the resolution adopted at St. Louis, the Executive Committee announced the appointment of A. L. Treadwell and A. A. Schaeffer as members of the Advisory Board to serve four years.

### *Committee reports*

Since the last meeting, President Kofoid appointed S. I. Kornhauser as the representative of the Society in a cooperative movement with the Society of American Bacteriologists to attempt to standardize American dyes for biological purposes. Professor Kornhauser reported that some information had been collected by means of questionnaires at Woods Hole and elsewhere, and requested members to furnish information concerning their experience with American dyes in comparison with standard stains formerly imported. Doctor Kornhauser stated that he was ready to answer inquiries at any time regarding available

American dyes. The Society voted \$25.00 from its funds to defray the expenses incurred in this investigation.

*Conference concerning the relations of genetics to the biological societies*

Early in 1921 L. J. Cole and R. A. Emerson sent out a circular letter to the officers and members of the Executive Committees of the societies interested calling attention to a movement among the younger geneticists of the country towards the formation of a separate society and suggesting that representatives of the societies now dealing with genetics meet in conference to discuss possible solutions which would give the geneticists the unity they need without causing the formation of another special society.

The conference was held at the University Club in Chicago, Friday, June 3, 1921, and was attended by Allen (President), Cowles, Crocker, L. R. Jones and Trelease of the Botanists; Allee, Cole, Detlefsen, Guyer and F. R. Lillie of the Zoologists and Davis (President) of the Naturalists.

The conference adopted the following recommendations by general consent without a dissenting voice:

1. That the Division of Biology and Agriculture of the National Research Council call a meeting of officers or representatives of biological societies at Toronto to discuss the formation of a federation of biological societies.

2. That sections be formed in the Botanical Society of America and the American Society of Zoologists to have charge of the genetics programs of the societies beginning with the Toronto meeting, under the joint direction of an executive secretary from each society and a single chairman who will be chosen alternately from each society.

Further details in the development of this plan were referred to the secretaries of the two societies.

As a result of the first recommendation, on invitation of the Division of Biology and Agriculture, National Research Council, a conference of the presidents and secretaries of a number of biological organizations met at Toronto with representatives of the Division, on the afternoon preceding the meetings. The resolutions adopted by this conference were presented to the Society by F. R. Lillie, and approved by the Society. The resolutions follow:

*Resolved*, 1. That it is the sense of this conference that an intersociety conference should be called to study and report upon the feasibility of federation of the biological societies and to develop plans for the said federation.

2. That for the purpose of effecting such an organization, each society, and Sections F and G of the American Association for the Advancement of Science, be requested to designate its President and Secretary as members of an inter-

society council which shall be authorized, 1) to deal with all matters of common interest, such as pooling of programs, that are consistent with the existing regulations of the constituent societies, and, 2) to draw up proposals for a constitution and by-laws of a federation of the societies in question, and to present them for action at the next annual meeting.

Although no formal action was taken, it was understood that the conference raised by the adhering societies should be empowered to invite other organizations to join it later.

In accordance with the second recommendation of the Chicago Conference, the Genetics Sections of the two societies met together in Lecture Theatre 60 A of the Biological Building beginning on Wednesday morning, December 28. The sections were called to order by Dr. R. A. Emerson, Executive Secretary for the Botanists. He at once appointed a committee of three to nominate a chairman for the Toronto meeting. A. F. Blakeslee was nominated and duly elected, taking the chair at once.

In the afternoon, upon motion, the chair appointed L. J. Cole, R. A. Emerson, H. S. Jennings, A. F. Shull, and G. N. Collins a committee to formulate a plan of organization for the Genetics Sections to report on Friday morning.

In the Zoölogists business meeting Friday morning the following action was taken authorizing the formation of a Section in Genetics:

*Moved*, That authorization be given for the formation of a Genetics Section of the American Society of Zoölogists to coöperate with the similar section established in the Botanical Society of America.

It shall be understood that any member of the American Society of Zoölogists may become a member of the Genetics Section by indication of his desire to that effect.

Any member of the Society submitting a paper on genetics has the right to have it included in the program of the Genetics Section.

The Genetics Section may designate one of its members as a consulting member of the Executive Committee of the American Society of Zoölogists.

The Genetics Sections reconvened following the Business Meeting of the American Society of Zoölogists. The committee on organization submitted the following report:

*Articles of organization*

*Resolved:* That the Genetics Sections of the American Society of Zoölogists and the Botanical Society of America organize for the purpose of securing a closer coördination of genetic interests.

The membership shall consist of those members of the two Societies who shall indicate their desire to be affiliated with the Genetics Sections.

The following officers shall be elected at each annual meeting and shall take office at the close of the meeting.

1. A Chairman, to be chosen alternately from the Zoölogical Section and from the Botanical Section.

2. A Secretary, who may be chosen from either Section.

3. A Society Representative, who shall be chosen from the Section other than from that from which the Secretary is chosen.

These three officers shall constitute the Executive Committee of the Genetics Sections.

In addition to his usual duties, the Secretary shall, in consultation with the other members of the Executive Committee and with the Secretaries of the Societies, arrange the program of the meetings.

The Secretary and the Society Representative shall act as the representatives of the Genetics Sections to their respective Societies.

At the annual meeting the Chairman shall, in advance of the business meeting, appoint a Nominating Committee of three to nominate officers for the following year.

These articles were adopted as proposed.

Upon motion, the Chairman appointed the following a committee to nominate officers for the coming year as provided in the Articles of Organization: R. A. Emerson, G. H. Shull, Charles Zeleny.

This committee later announced as nominees:

For Chairman: H. S. JENNINGS.

For Secretary: L. J. COLE.

For Society Representative: B. M. DAVIS.

These were duly elected.

The Secretary reported on the condition of the American Genetics Association and the needs of the *Journal of Heredity*, and stated that efforts would be made to hold a conference between the Executive Committee of the Genetics Sections and the Council of the American Genetics Association to see what steps can be taken for their mutual benefit.

This transcript of the proceedings of the Genetics Sections, together with the report of the papers presented which will be



found elsewhere in these proceedings, was furnished by the Secretary of the Sections, Dr. L. J. Cole.

The following resolution, growing out of the conference concerning a similar organization of parasitologists within the Society was presented and approved:

*Resolved*, That the Parasitologists assembled at Toronto request the officers representing the Society in the proposed conference on federation of biological societies, and, further, instruct their special representative, should the conference admit one, to urgently request, that the conference arrange for membership in the Section of Parasitology of persons who do not meet the present requirements for membership in the Society of Zoölogists.

The parasitologists appointed C. A. Kofoid as their representative, with B. H. Ransom as alternate.

In accordance with action taken at the last meeting, President Kofoid had appointed V. E. Shelford a delegate from the American Society of Zoölogists to the National Conference on Parks held in Des Moines, Iowa, January 10, 11 and 12, 1921. Dr. Shelford submitted the following report:

While a small number of scientific societies were represented, the conference was well attended, especially by those interested in natural parks for recreation purposes. Their aim is to secure more parks and protect existing ones. Very few of the existing parks and preserves are free from liability to extensive modification through recreation activities, scientific forestry, fires, or exploitation. Even the National Parks must be watched and defended. There are now only a few areas aside from the National Parks which have been set aside with the intention that they should be left in a natural state. Most areas have been and probably will continue to be set aside primarily as recreation parks, or as forest preserves. The main business of those interested in areas to be held in an original state, must of necessity be to get areas set aside within these forest preserves and parks.

The following was made evident by the conference:

1. That the forces interested in the establishment of natural parks and forest preserves for recreation purposes—to make "better citizens through contact with nature" are well organized, and are probably the strongest force operating to secure more parks and protect existing ones.

2. Science has left them quite ignorant of its needs for natural areas and of the practical significance of scientific results which may accrue from study of natural areas. They welcome the idea of biological study "as a further argument for natural tracts."

3. They are, however, without constructive plans of management of the smaller tracts which will insure them against destruction from over use as recreation

parks. Such plans of management must be based on knowledge of plant and animal ecology which they do not possess.

4. They are engaged in drafting legislation and in advising legislators without the counsel of those interested in preserves for research purposes.

5. It is incumbent upon scientific societies, museums, and Universities to organize and to provide funds which will serve the following purposes: (a) to place information as to the scientific uses, and scientific management of natural areas, into the hands of those individuals and organization working for the preservation of natural conditions (b) to make possible the representation of scientific needs before legislative bodies and officials (c) to provide for furthering the wise selection of new areas, and (d) to make existing areas accessible to scientists by the publication of lists and guide books.

### *Communications from other organizations*

Eugene C. Bingham, of Lafayette College, Chairman of the Metric Committee of the American Chemical Society, urged all Zoölogists to assist in the establishment of metric units for scientific materials, especially in the matter of ordering chemicals by metric units.

Robert M. Yerkes, of the Research Information Service, spoke briefly concerning the purpose and possibilities of that Service. An abstract of his account is published in the Proceedings.

From the Ecological Society of America, December 28, 1921:

#### *To the American Society of Zoölogists:*

For several years past the Ecological Society through the present committee has been locating and briefly describing areas in which the original flora and fauna are little disturbed and which are accordingly suitable for scientific research. Some knowledge regarding about 1000 areas in the United States and Canada is on hand, but the degree of modification from the original state must be estimated for each area. It has been proposed to use letters to designate the condition of the flora and figures to designate the condition of the fauna. It has been suggested that there is only one area in the United States which is really in its original condition. This is due to the general destruction of large game.

It is regarded as highly desirable to press the work to completion and publish it at an early date. The funds available from the Ecological Society and from the National Research Council cannot provide for circulating the necessary questionnaires at an early date. The Ecological Society accordingly authorized the committee to request the American Society of Zoölogists to provide for the work of classifying areas from the standpoint of animals, funds up to \$25.00.

The Society of Zoölogists will be given full credit for this work if the request is granted.

Respectfully submitted by

V. E. SHELFORD,  
*Senior Chairman.*

The Society voted that \$25.00 from its funds be placed to the credit of V. E. Shelford for this work, as requested.

*General resolutions*

The following resolution was adopted and ordered sent to the appropriate officials:

The American Society of Zoölogists, understanding that there is a temporary suspension of certain scientific publications of the U. S. Government, including the Journal of Agricultural Research, the Experiment Station Record, and the Monthly Weather Reports, desires to put on record its very high appreciation of these journals and of their great national and international importance in the field of natural science and would respectfully urge their resumption at as early a date as possible.

The following resolution was adopted:

*Resolved*, That the Secretary express to President the of the University of Toronto and the local committee on arrangements the high appreciation of the American Society of Zoölogists for the splendid facilities afforded this meeting and for the cordial hospitality shown the members attending.

PROGRAM

WEDNESDAY, DECEMBER 28

- 10:00 A.M. Section A, Lecture Theatre 14, Biological Building. Papers dealing with Embryology, Cytology, and Comparative Anatomy; seven papers presented in full, five of which were discussed. Attendance, 75.
- 10:00 A.M. Section B, Lecture Theatre 60A, Biological Building. Papers on Evolution and Genetics from the Botanical Society of America. Attendance, about 75.
- 2:00 P.M. Section B continued with papers from the American Society of Zoölogists. Attendance, 125 to 150.
- 5:00 P.M. Meeting Executive Committee.
- 8:30 P.M. Public Address before the entire American Association in Convocation Hall by Professor William Bateson, who had been brought over from England on the initiative of the American Society of Zoölogists in coöperation with the American Association. President Kofoed introduced the speaker of the evening.
- 10:00 P.M. Biological Smoker, Hart House. The smoker was arranged by the American Society of Zoölogists. All interested persons were invited to attend. Smokes and cold refreshments were furnished by the Local Committee. Hundreds present.

## THURSDAY, DECEMBER 29

- 9:40 A.M. Biological Building, Lecture Theatre 14; Joint meeting with the Ecological Society of America for the presentation and discussion of papers on Ecology and Zoogeography. Eleven papers were presented in full, seven of which were followed by discussion. President S. A. Forbes, of the Ecologists, presided during most of this session. Attendance, 90.
- 2:00 P.M. Section A, Parasitology, Lecture Theatre 14. President Kofoid, presiding. Herbert Rand, Secretary. Eight papers were presented in full. By vote of the Society, B. H. Ransom was given as much time as he needed to show two reels of films for his paper on "A new venture in the field of practical parasitology." At the close of the session, the members present spent about 45 minutes in discussing the problem of organization. The resolution adopted is recorded in the proceedings of the business meeting. Attendance, 45.
- Section B. General and Comparative Physiology. Lecture Theatre 60 A. Vice-President Treadwell presiding. Nine papers presented in full, four of which were followed by discussion. The section voted to receive papers by E. Horne Craigie and W. H. Longley which had arrived too late for inclusion in the general program. Attendance, 24.
- 5:00 P.M. Meeting Executive Committee.

## FRIDAY, DECEMBER 30

- 9:30 A.M. Business Session, Lecture Theatre 14. Maximum attendance, about 75. In addition to the business, papers were presented at this session by Robert M. Yerkes. (No. J. 1) and by E. E. Prince, Dominion Commissioner of Fisheries (No. J. 2). The latter was followed by discussion.
- 2:15 P.M. Medical Building, Lecture Theatre 19. Zoölogists' Symposium on *Orthogenesis*. This was participated in by Lawrence Henderson, C. B. Lipman, M. F. Guyer, H. F. Osborn, J. C. Merriam, and William Bateson with some discussion from the chair and from the floor. Attendance, over 220.
- 7:45 P.M. Zoölogists' Dinner in the dining Hall at Knox College, Address, "The Outlook in Genetics" (illustrated), by William Bateson. Attendance, 112.

In coöperation with Botanical Society of America, the American Society of Naturalists, the Ecological Society of America and other interested societies, the Zoölogists participated in a bulletin service whereby members could keep track of the progress of the programs in related societies. The service was arranged by and under the direction of J. R. Schramm, Secretary of the Botanical Society.

## LIST OF TITLES

The following titles, contributed for the program, have been grouped and arranged in accordance with rules accepted by the Society.

*Papers marked with an asterisk were read by title.*

## A. EVOLUTION AND GENETICS

*Papers from the Botanical Society of America*

1. The synthesis of full coloration in Phlox. (4 min.) James P. Kelly.
2. The inheritance of shape in the fruit of the summer squash. (Lantern; 5 min.) Edmund W. Sinnott.
3. Correlated inheritance in wheat of winter-spring habit of growth and rust resistance. (Charts; 10 min.) Olaf S. Aamodt.
4. The linkage relations of the factors for shrunken endosperm *Sh sh*, waxy endosperm *Wx wx* and the aleurone color factor *C c* and *I i* in maize. (Lantern; 15 min.) C. B. Hutchison.
5. Crossing over in F1 maize-teosinte hybrids. (Lantern, 5 min.) L. F. Randolph.
6. Mexican wild relatives of maize. (Lantern, 20 min.) G. N. Collins.
7. Studies of somatic mutations in variegated maize pericarp: I. Relative frequency of dominant somatic mutations in homozygous and in heterozygous variegated pericarp. (Lantern; 15 min.) R. A. Emerson. II. Frequency of mutation in relation to development. (Lantern, 10 min.) W. H. Eyster and E. G. Anderson. III. An interpretation. (Lantern, 10 min.) E. G. Anderson and M. Demerec.
8. Studies on the pollen tubes and abortive ovules of the Globe mutant of *Datura*. (10 min.) J. T. Buchholz and A. F. Blakeslee.
9. The effect of a single gene as compared with that of a single chromosome upon the gross structure of *Datura*. (Lantern; 10 min.) E. W. Sinnott and A. F. Blakeslee.
10. Three new mutations in *Oenothera lamarckiana*. (Lantern; 12 min.) George H. Shull.
11. Variation and mutation in *Pestalozzia Guepini* Desm. (Lantern; 8 min.) Carl D. LaRue.
12. Inheritance of flower types and fertility in the strawberry. (10 min.) W. D. Valleau.
13. The accumulative effect upon a pure line of wheat, of ten generations of growth under diverse conditions of soil fertility. (10 min.) C. H. Myers.
14. The immediate effect of foreign pollen upon the kernel development of maize. (15 min.) T. A. Kisselbach.

## A'. EVOLUTION AND GENETICS

*Papers from the American Society of Zoologists*

1. Relative nuclear volume and the life cycle of *Hydatina senta*. (Lantern.) A. Franklin Shull, University of Michigan.
2. The action of alcohol upon germinal material. (Lantern.) E. Carlton MacDowell, Cold Spring Harbor.

3. Parallelism in Ascidians, with a new conception of organic structure. A. G. Huntsman, University of Toronto.
- \*4. Effect of temperature upon the development of the eye of *variable*, a mutant from *Drosophila hydei*. Roscoe R. Hyde, The Johns Hopkins University.
5. Heritable effects of chemically differing media on the fission rate of *Paramecium caudatum*. A. R. Middleton, University of Louisville.
6. Heredity of resistance to tuberculosis in guinea-pigs. (Lantern.) Sewall Wright and Paul A. Lewis, Bureau of Animal Industry and Phipps Institute.
- \*7. Data on the inheritance of spurs in the female fowl. H. D. Goodale, Massachusetts Agricultural Experiment Station.
8. Heredity of build. (Lantern.) Charles B. Davenport, Cold Spring Harbor.
9. Effective reverse selection in bar eye of *Drosophila* due to the appearance of mutations. (Lantern.) Charles Zeleny, University of Illinois.
10. Genetic mosaics and ontogenetic abnormalities in the parasitic wasp, *Hadrobracon*. (Charts.) P. W. Whiting, University of Iowa.
11. Olive, a mutation in *Colias philodice*. John H. Gerould, Dartmouth College.
12. Incomplete synapsis of chromosomes and its possible relation to linkage variations. (Lantern.) C. W. Metz, Carnegie Institution of Washington.
13. A cross in guinea-pigs best explained by assuming 75 per cent crossing over. (Lantern.) Herman L. Ibsen, Kansas State Agricultural College.
14. A linkage diagram of nine factors for color patterns in *Apotettix eurycephalus* Hancock. Robert K. Nabours, Kansas Agricultural Experiment Station.
15. Genetic analysis of low crossover stock, produced by selection. J. A. Detlefsen and L. S. Clemente, University of Illinois.
16. Ten years with the self-fertilized line of *Lymnaea columella* Say. Harold Sellers Colton, University of Pennsylvania.
17. Results of eight years of inbreeding of Rhode Island Red fowls. (Lantern.) L. J. Cole and J. G. Halpin, University of Wisconsin.
- \*18. Inheritance of color in the domestic turkey (continued). W. R. B. Robertson, University of Kansas.
- \*19. Orthogenesis of non-homochromic pigmentation in Chromodorids. W. J. Grozier, Rutgers College.
- \*20. Breeding experiments with the viviparous teleosts, *Xiphophorus helleri* and *Platyopocilus maculatus*. (Günth.) A. W. Bellamy, University of Chicago.
21. The elimination of the sex-chromosome by X-rays: A modification of the germ plasma produced by an external agent. James W. Mavor, Union College.
- \*22. Hybrid vigor, hybrid weakness and the chromosome mechanism of heredity. An experimental analysis of the physiology of heredity in the reciprocal crosses between two closely associated species of sea-urchins, *Strongylocentrotus purpuratus* and *S. franciscanus*. H. H. Newman, University of Chicago.
- \*23. Changes in egg production at the Massachusetts Agricultural Experiment Station. H. D. Goodale, Massachusetts Agricultural Experiment Station.

24. Variations in the number of vertebrae and other segmental characters of fishes correlated with the temperature of the water during development. Carl L. Hubbs, University of Michigan. (Introduced by L. V. Heilbrunn.)
25. (Received by vote of the section.) A lethal gene which changes the order of the loci in the chromosome map. H. J. Muller, University of Texas.

B. EMBRYOLOGY

1. Cell behavior in tissue culture. (Lantern.) H. B. Goodrich, Wesleyan University.
- \*2. Experiments with *Necturus* and axolotl thyroids. W. W. Swingle, Yale University.
- \*3. Chorionic fusion and augmented twinning in the human tube. Leslie B. Arey, Northwestern University Medical School.
4. The influence of thyroid gland feeding upon tadpoles from which the thyroid gland and the buccal anlage of the hypophysis have been removed. Ben-net M. Allen, University of Kansas.

C. CYTOLOGY

1. A determination of the degree of constancy in the nuclei of certain organs in *Hydatina senta*. H. J. Van Cleave, University of Illinois.
2. Further studies in the cytology of *Asolabis maritima* Bon. (Lantern.) S. I. Kornhauser, Denison University.
- \*3. The occurrence of telosynapsis in the male germ cells of *Leptocoris trivittatus* Say (Hemiptera). Harry B. Yocum, University of Oregon.
- \*4. Studies on the cells of sheep with special reference to spermatogenesis, oogenesis and sex-determination. (Lantern.) J. E. Wodsdalek, University of Idaho.
5. The structure and division of *Trichomonas augusta*, Alexeieff. D. H. Wenrich, University of Pennsylvania.
6. Multinucleated germ cells in the Columbian ground squirrel. (Lantern.) J. E. Wodsdalek, University of Idaho.
- \*7. Seasonal changes in the testis of *Gambusia affinis*, the top-minnow. S. W. Geiser, The Johns Hopkins University. (Introduced by S. O. Mast.)
- \*8. Changes in the vaginal epithelium of the guinea pig during the oestrous cycle. R. M. Selle, University of California. (Introduced by J. A. Long.)
- \*9. (By vote of the Section, the following papers were received, ordered placed on the program and abstracts were ordered printed.)  
The spermatogenesis of man, Theophilus S. Painter, University of Texas.
- \*10. The Spermatogenesis of the opossum. Theophilus S. Painter.

D. COMPARATIVE ANATOMY

- \*1. Metamorphic changes in the digestive system in *Rana pipiens* and *Ambystoma tigrinum*. Albert Kuntz, St. Louis University School of Medicine.
2. The skull of *Syngnathus fuscus*. James E. Kindred, Western Reserve University.

- \*3. The thyroid glands of perennibranchiate Amphibians. W. W. Swingle, Yale University.
- \*4. On the muscular system of *Ornithodoros moubata* (Murray). E. Melville Du Porte, Macdonald College, McGill University.
- 5. On the structure and development of a fat body or gland in the rat. J. A. Long, University of California.
- \*6. Relation of growth to metamorphosis in the lungless salamander, *Eurycea bislineata*. Inez Whipple Wilder, Smith College.
- \*7. Degeneration in the pelvis of the female pocket gopher, *Geomys bursarius*. Frederick L. Hisaw, Kansas State Agricultural College. (Introduced by J. E. Ackert.)

## E. ECOLOGY AND ZOOGEOGRAPHY

- 1. Chemical composition of some fresh-water organisms. C. Juday, University of Wisconsin and Wisconsin Geological and Natural History Survey.
- 2. Some adaptations of mayfly nymphs to swift streams. C. S. Dodds and F. L. Hisaw, West Virginia University.
- 3. Some physical factors related to the distribution of littoral invertebrates. W. C. Allee, University of Chicago.
- 4. Ecology of parasites of lake fishes. A. S. Pearse, University of Wisconsin.
- 5. The eggs of the frogs, tree frogs and toads of Okefinokee Swamp, Georgia. A. H. Wright, Cornell University.
- 6. Further studies on the circulation of water in the Bay of Fundy and the Gulf of Maine. James W. Mavor, Union College.
- 7. The effect of temperature in limiting the geographical range of invertebrates of the Woods Hole littoral. W. C. Allee, University of Chicago.
- 8. Hydrogen ion concentration of Chesapeake bay water. R. P. Cowles and A. M. Schwitalla, S.J., The Johns Hopkins University. (Introduced by S. O. Mast.)
- \*9. *Canuella canadensis* and other Canadian copepods. A. Willey, McGill University. (Introduced by A. G. Huntsman.)
- 10. Notes on the Crocodilia of British Guiana. A. M. Reese, University of West Virginia.
- 11. A noteworthy adaptive modification of the webs of a Siamese Agriopid spider. H. E. Crampton.
- 12. On the distribution and ecology of the species of *Partula* inhabiting the Mariana Islands of the Western Pacific Ocean. H. E. Crampton, Barnard College.

## F. GENERAL ZOÖLOGY

- 1. On the rate of growth of *Teredo* and *Xylotrya*. (Lantern.) Thurlow C. Nelson, Rutgers College and the New Jersey State Board of Shellfisheries.
- \*2. Observations on sex in the top-minnow, *Gambusia affinis*. S. W. Geiser, The Johns Hopkins University. (Introduced by S. O. Mast.)



## G. PROTOZOOLOGY

- \*1. Trophic and reproductive chromatin in the ciliate infusoria compared with similar conditions in other protozoa. M. M. Metcalf.
- \*2. Some effects of conjugation in the life history of *Spathidium spathula*. Lorande Loss Woodruff and Hope Spencer, Yale University.

## H. PARASITOLOGY

(All workers in America who have recently published work in parasitology are invited to participate in the parasitology program at Toronto on the same terms as members of the Society.)

- \*1. The parasitic Protozoa of *Blatta orientalis* and their value as the material for use in class of parasitic Protozoa. R. Kudo, University of Illinois.
- 2. A preliminary report of the activities of infective hookworm larvae in the soil. William W. Cort and Donald L. Augustine, The Johns Hopkins University.
- \*3. Notes on North American blood flukes. G. A. MacCallum, Baltimore.
- \*4. A study of *Diphyllbothrium ova*. Meyer Wigdor.
- 5. A new genus of Trematodes from the white-bass. H. J. Van Cleave, University of Illinois.
- \*6. Apparent racial immunity to certain Nematode infections. Benjamin Schwartz and Marcos A. Tubangui, University of the Philippines.
- 7. Intestinal protozoan infections as an index of personal hygiene and institutional sanitation. William C. Boeck, University of Wyoming.
- 8. *Ophiotaenia testudo* nov. spec. from *Amyda* (*Trionyx*) *spinifera*. Thomas Byrd Magath, Mayo Clinic.
- 9. Otacariasis in the fox. Wm. A. Riley, University of Minnesota.
- 10. A new venture in the field of practical parasitology. (Motion pictures.) B. H. Ransom, U. S. Bureau of Animal Industry.
- \*11. The host-parasite method of investigation and some problems to which it gives approach. M. M. Metcalf.
- \*12. The rôle of domestic chickens and pigs in the spread of hookworm disease. James E. Ackert, Kansas State Agricultural College.
- \*13. A study of the resistance of different hosts to Trypanosome infections. W. H. and L. G. Taliaferro.
- \*14. The anomalies in the group of blood infesting Trematodes. Horace W. Stunkard, New York University.
- 15. Some additional results obtained in the study of infectious anemia of horses. John W. Scott, University of Wyoming.
- \*16. Sporulation and development of the cysts in a new species of Myxosporidia, *Lentospora ovalis*. H. S. Davis, University of Florida.
- \*17. On the migration of the sheep lung worm, *Dictyocaulus filaria*. John E. Guberlet, Oklahoma Agricultural Experiment Station.
- 18. Studies on *Spirochaeta duttoni* in the tissues of its invertebrate host *Ornithodoros moubata*. E. Melville DuPorte, McGill University.
- \*19. A little-worked source for parasite material. M. M. Metcalf.
- 20. A coelomic Coccidian of *Tribolium*. Wm. A. Riley and Laurene Krogh, University of Minnesota.

- \*21. On *Leptotheca ohlmacheri*, a Myxosporidian parasitic in frogs. R. Kudo, University of Illinois.
- \*22. Notes on two new species of Holostomidae. John E. Guberlet, Oklahoma Agricultural Experiment Station.
- \*23. Phases in the life history of a Holostome, *Cyathocotyle orintalis*, nov. spec., with notes on the excretory system of the larva. Ernest Carroll Faust, Peking Union Medical College, Peking, China.

## I. COMPARATIVE AND GENERAL PHYSIOLOGY

- \*1. Mutual reactions of cell-bodies and pseudopodial fragments of *Diffugia*. Wm. A. Kepner and B. D. Reynolds, University of Virginia.
- \*2. Reactions to light of the earthworm, *Lumbricus terrestris*. Walter N. Hess, DePauw University.
- 3. The rate of metabolism of *Paramecium* with controlled bacterial food supply. Geo. T. Hargitt and Ruth L. Phillips, Syracuse University.
- \*4. The feeding reactions of *Ambystoma tigrinum* (Green). Albert Kuntz and Jose Zozaya, St. Louis University School of Medicine.
- 5. The rôle of the nervous system in the locomotion and regeneration of marine polyclads. J. M. D. Olmsted, University of Toronto.
- 6. A quantitative study of tarsal sensitivity to solutions of saccharose in the Red Admiral butterfly, *Pyraeas atalanta* (Linn). Dwight E. Minnich, University of Minnesota.
- \*7. Micro-Winkler method for quantitative determination of dissolved oxygen. E. J. Lund, University of Minnesota.
- 8. A contribution to the colloid chemistry of protoplasm. L. V. Heilbrunn, University of Michigan.
- \*9. Transplantation of the pars nervosa of the pituitary. W. W. Swingle, Osborn Zoological Laboratory, Yale University.
- \*10. Phototaxis in the robber-fly, *Proctacanthus*. S. O. Mast, The Johns Hopkins University.
- 11. Light and growth in animals. A. G. Huntsman and F. Fraser, University of Toronto.
- \*12. "Reversal of inhibition" in insects, by atropine. W. J. Crozier, Rutgers College.
- \*13. Electrical polarity in *Obelia*. E. J. Lund, University of Minnesota.
- \*14. Circus movements in negatively heliotropic *Limax*. W. J. Crozier, Rutgers College, and W. H. Cole, Lake Forest College.
- \*15. The effect of locomotion in *Ameba*. I. Reactions to localized stimulation. J. Graham Edwards, The Johns Hopkins University. (Introduced by S. O. Mast.)
- \*16. A quantitative study of the reactions to light in *Ameba*. Harry T. Folger, The Johns Hopkins University. (Introduced by S. O. Mast.)
- 17. The influence of temperature on the rate of locomotion in *Ameba*. Alphonse M. Schwitalla, St. Louis University. (Introduced by S. O. Mast.)  
(Papers received and abstracts ordered printed by vote of the Section)
- 18. The reflex produced by chemical stimulation of the deeper respiratory passages. E. Horne Craigie, University of Toronto.

19. The instincts and adaptations of several species of Tortugas fishes. W. H. Longley, Goucher College.
- \*20. Hen feathering in male fowls by feeding thyroid. Benjamin Horning and Harry Beal Torrey, University of Oregon Medical School.

J. UNCLASSIFIED PAPERS

1. The National Research Council Information Service. Robert M. Yerkes.
2. A Rare Example of Fish Teratology in Bicephalous Shark. E. E. Prince, Dominion Commissioner of Fisheries. (Introduced by W. C. Allee.)

SYMPOSIUM ON ORTHOGENESIS

1. Orthogenesis from the Standpoint of the Biochemist. L. J. Henderson.
2. Orthogenesis in Bacteria. C. B. Lipman.
3. Orthogenesis in Serological Reactions. M. F. Guyer.
4. Orthogenesis as Observed from Paleontological Evidence. H. F. Osborn.  
(Discussion by J. C. Merriam).
5. General Discussion. William Bateson.

EXHIBITS

1. Charts illustrating the elimination of the sex-chromosome from *Drosophila* by X-rays. James W. Mavor, Union College.
2. Charts and Diagrams illustrating the circulation of water in the Bay of Fundy and the Gulf of Maine. James W. Mavor, Union College.
3. A chart showing drift bottles which have crossed the Atlantic from the Canadian Coast since the summer of 1919. James W. Mavor, Union College.
4. The nuclei of the vitellaria of *hydatina*. H. J. Van Cleave, University of Illinois.
5. A demonstration of decerebrate rigidity, flexion and crossed extension reflexes, reciprocal innervation and inhibition in the cat. J. M. D. Olmsted and W. P. Warner, University of Toronto.
6. A demonstration of *Paramecium calkinsi*, sp. nov. L. L. Woodruff, Yale University.



## ABSTRACTS

### A. EVOLUTION AND GENETICS

#### PAPERS FROM THE BOTANICAL SOCIETY OF AMERICA

1. *The synthesis of full-coloration in Phlox.* JAMES P. KELLY, State College, Pa.

In the issue of *Genetics* for March, 1920, the writer published data on color of flower blade in *Phlox Drummondii*. Certain  $F_1$  purples that were full-colored and self-colored gave an  $F_2$  group of several blade types, i.e., full-colored selfs, lighter colored selfs, stippled dusky type, and white-bladed type. The  $F_2$  results showed that the second and third types never gave rise to each other in progeny, while the first might throw out the second and third types besides repeating itself. Such analysis led to inference that full color was due to presence together of second and third types, or, rather of the genes for these types. In the present communication the writer reports the synthesis of full-colored selfs by the putting together in hybridization of the second and third types mentioned above.

2. *Variation and mutation in Pestalozzia Guelpini Desm.* CARL D. LA RUE.

The length of spores of *Pestalozzia Guelpini* varies from generation to generation. A generation with long spores is followed by two or three generations with spores of reduced size, after which another long-spored generation appears. Fluctuations of a similar type have been found by other workers and have usually been attributed to environmental influences. In *Pestalozzia* the variations appear to be independent of fluctuations in environmental factors. They are likewise independent of the composition of the nutrient medium. One strain of *Pestalozzia Guelpini* gave rise to a mutation differing markedly from the parent strain both in spore characters and in vegetative development. From this mutation another originated with vegetative characters like those of the original strain but with spores identical in size and appearance with those of the first mutant. In another strain a mutation arose originated which differs from the parent strain in vegetative characters only.

3. *Correlated inheritance in wheat of winter-spring habit of growth and rust resistance.* (Charts.) OLAF S. AAMODT. Cooperative investigations between the Office of Cereal Investigations, U. S. Dept. of Agriculture, and the Department of Agriculture of the University of Minnesota.

The study is one of the steps leading toward the production of a rust-resistant spring wheat. The parental varieties, Kanred and Marquis, belong to *Triticum vulgare*. Kanred, a winter wheat, is resistant to several biologic forms of *Puccinia graminis tritici* (Eriks. & Henn.) to which Marquis, a spring wheat, is susceptible.

At University Farm, St. Paul, Minnesota, the Kanred parent, when planted in the spring, produces only an occasional head late in the season which fails to set seed. The  $F_2$  Kanred-Marquis cross was planted in the spring and the plants

were placed in nine groups according to time of heading. Seven of these groups set seed and were tested in  $F_2$ . All individuals of the earlier heading  $F_2$  group bred true for spring habit of growth. In the other six groups in  $F_2$  the percentage of spring plants was in direct relation to the time of heading of the  $F_2$  group.

$F_3$  seedlings of each  $F_2$  group were inoculated, in the greenhouse, with a single known biologic form of rust. The segregation approximated a ratio of three resistant to one susceptible plant. The ratio of resistant to susceptible plants was approximately the same for all heading periods. Preliminary tests indicate that the reaction to several biologic forms was inherited as a single genetic factor.

4. *Studies on the pollen tubes and abortive ovules of the globe mutant of Datura.*

J. T. BUCHHOLZ and A. F. BLAKESLEE, University of Arkansas.

A technique of dissection was developed which enables us to stain and count nearly all of the pollen tubes within the nutritive tissue of the style. The pollen from Globe produces upon germination two groups of pollen tubes, those that grow rapidly, and others that grow more slowly, as indicated by the bimodal curve of pollen tube distribution in the style. A large number of abortive ovules are produced in Globe selfed, in Globe Normal, and quite a few even in Normal  $\times$  Globe, the latter indicating that probably a number of pollen tubes transmitting the Globe character actually reach the ovary above the small number of Globe seeds produced by this cross. Counts have been made of the pollen applied, the residue of ungerminated pollen grains on stigma and the residue of pollen tubes remaining in the style after abscission, from which the number of pollen tubes entering the ovary may be estimated.

5. *The linkage relations of the factors for shrunken endosperm  $Sh\ sh$ , waxy endosperm  $Wx\ wx$ , and the aleurone color factors  $C\ c$  and  $I\ i$  in maize.* C. B. HUTCHISON.

It has been shown previously by other investigators and by the writer that the factors for waxy endosperm  $Wx\ wx$  and shrunken endosperm  $Sh\ sh$  and two of the factors concerned in aleurone color,  $C\ c$  and  $I\ i$  in maize are linked. Results are here reported from two backcross experiments, the one involving shrunken and waxy endosperm and the  $I\ i$  color pair, the other shrunken and waxy endosperm and the  $C\ c$  color pair, which are interpreted as showing that the order of the genes are  $I\ Sh$  and  $Wx$ , and  $C\ Sh\ Wx$ . The per cent of crossovers between  $I$  and  $Sh$  is practically the same as that between  $C$  and  $Sh$ .  $C$  and  $I$  must therefore be allelomorphic or very closely linked.

6. *Studies of somatic mutations in variegated maize pericarp.* I. R. A. EMERSON: Relative frequency of dominant somatic mutations in homozygous and in heterozygous variegated pericarp. 15 minutes. (Lantern.) II. W. H. EYSTER and E. G. ANDERSON: Frequency of mutation in relation to development. 10 minutes. (Lantern.) III. E. G. ANDERSON and M. DEMEREC: An interpretation. 10 minutes. (Lantern.)

It has been shown previously that there occur, in races of maize with variegated pericarp, somatic mutations from the recessive variegated type to the dominant self-color type, the areas affected varying from a part of a seed to the whole ear. Two types, one inherited and the other not inherited, have been recognized. It has been pointed out that homozygous material, having the recessive variegation factor duplex, might be expected to mutate twice as frequently as hetero-

zygous material having that factor simplex. Results are here reported, showing on the contrary that somatic mutations occur consistently with greater frequency when the variegation factor is simplex than when it is duplex. Tabulation of progenies derived from a gene for variegation shows a high frequency of mutation in both directions, i.e., to self-color and to white. A gene for light variegation mutates frequently to white but rarely to self-color. A gene for dark variegation mutates rarely to white but frequently to self-color. The self-color derivatives show occasional reverse mutations to variegated. The mutations from variegated to self-colored affect both the germ cells and somatic tissue. The effect of a mutation on the somatic tissue of pericarp and glumes is visible as a colored area. The small colored areas are supposed to be due to mutations occurring relatively late in ontogeny, earlier mutations giving rise to larger colored areas. If mutability remains constant the frequency of observed mutant areas of each size should be roughly proportional to the relative number of cells present at the respective stages at which the mutations occurred. Tabulations of about 45,000 observed mutant areas of different sizes show a great increase in mutability in the later stages. This is believed to be causally related to increasing differentiation.

7. *The inheritance of shape in the summer squash.* EDMUND W. SINNOTT.  
(No abstract received.)

8. *Three new mutations in Oenothera Lamarckiana.* GEORGE H. SHULL.

The origin and characteristics of the following new mutations are described: 1) *Oenothera Lamarckiana* mut. *funifolia* has strongly revolute leaves and is regarded as a parallel mutation in relation to *Oenothera pratincola* mut. *formosa* Bartlett. 2) *Oenothera Lamarckiana* mut. *pervirens* differs from the parent in having stems and buds wholly devoid of red pigmentation. 3) *Oenothera Lamarckiana* mut. *vetaurea* has flower color modified to a pale old gold color, the only previous departure in flower color in the *Oenotheras* having been to a pale yellowish white known as *sulfurea*. All three of these mutations are recessive to the parental type and at least (1) and (3) originated as new gene-mutations. Mut. *pervirens* may possibly have been produced as result of a crossover. More papers dealing with plant genetics will be added later.

#### A'. PAPERS FROM THE AMERICAN SOCIETY OF ZOÖLOGISTS

1. *Relative nuclear volume and the life-cycle of Hydatina senta.* (Lantern.)  
A. FRANKLIN SHULL, University of Michigan.

The ratio of nuclear volume to cytoplasmic volume, held by many biologists to be of importance in relation to regeneration, age, embryonic development, general cell physiology, sex, and other phenomena, was regarded by Hertwig, Isakowitch, Papanicolau and others as determining the form of the life-cycle in animals which reproduce alternately by parthenogenesis and bisexual reproduction. The rotifer *Hydatina* offers unusual material for testing this hypothesis. If the theory is correct, the relative nuclear volume should change to correspond with the periodicity of male-production that exists in most lines of this rotifer. Also since male-producers are more common in the middle of a family than at either the beginning or end of it, the nuclear volume should be relatively greater

in middle-aged females than in either young or old adults. Furthermore, those environmental agents (chemical substances, manure solution, food, etc.) which alter the life-cycle should also alter the relative nuclear volume. While significant changes in the volume of the nucleus have been discovered, these changes do not accord with the theory. The relative nuclear volumes in the yolk gland increased during a series of generations, while those of the stomach-intestine decreased during the latter half of the same period. Relative nuclear volume of the yolk gland increased throughout the life of the individual, instead of decreasing after middle age as the theory requires. Manure solution altered the nuclear volume of the stomach-intestine in the direction opposite to that required by the theory, but did not effect the other tissues studied. Female-producers and male-producers appeared to have equal nuclear volumes.

2. *The action of alcohol upon germinal material.* (Lantern.) E. CARLETON MACDOWELL, Station for Experimental Evolution, Cold Spring Harbor, Long Island, New York.

This paper presents a summary of various series of data obtained from white rats heavily dosed with alcohol fumes. Four groups of animals are considered: 1) those treated; 2) their untreated children; 3) their treated children; 4) their untreated children's untreated children. Judged by their behavior in a circular maze and by the size of their litters, all four groups show a small amount of inferiority in comparison with their respective controls. This result appears to support the interpretation that the alcohol has directly modified germinal material.

If the numbers of litters produced in the same periods by the tests and their respective controls are compared, it appears that the treated rats produced only 32 of the expected 91 litters, or 35 per cent of the controls' production. The treated offspring of these treated rats produced 65 per cent of the expected number; the untreated offspring of the treated rats produced 33 per cent more litters than expected and the untreated grandchildren produced 55 per cent more litters than expected. The treated rats grew more slowly than their controls; their treated children about equaled their controls in growth and weight; their untreated children were heavier than their controls and their untreated grandchildren showed a less positive tendency to surpass their controls. The results from weight and the number of litters appear to show that the alcohol has acted as a selective agent upon a series of genetic differences present in the original animals.

3. *Parallelism in Ascidians, with a new conception of organic structure.* A. G. HUNTSMAN, University of Toronto.

Ascidians in general and the family Styelidae in particular offer a very good example of parallelism in the evolution of body-form. In that family the outstanding case is the repeated appearance in distinct genera of a peculiar stalked type. The interpretation offered is that the lines along which evolution has passed have been limited, not by selection, but by the structure of the ancestral germ-plasm. A working conception of the structure of living matter is needed. At present we have knowledge, 1) of the visible structure and, 2) of the chemistry of decomposition products of living matter. These may be loosely connected by considering that corresponding with the definite unity of a living mass there is an



enormous, complex, and ever-changing molecule, which is the foundational and essential part of the mass, and with which are more or less closely associated the numerous simple 'lifeless' molecules of which we have knowledge.

4. *Effect of temperature upon the development of the eye of (variable), a mutant from Drosophila hydei.* ROSCOE R. HYDE, Johns Hopkins University.

The eggs from a new eye mutant, variable from *Drosophila hydei* (Sturtevant) when developed under warm, dry conditions give rise to flies with very small eyes. In fact, the eyes of some individuals may be entirely missing. When the eggs are subjected to a temperature below that of the living room and well supplied with moist food, the flies that emerge have large eyes, indistinguishable from the wild type. The eyes of the wild stock from which mutant came are not modified by changes in temperature. The new eye character is a non sex-linked recessive and shows linkage with scarlet, another mutant of this species. The two characters show crossing over in the female but not in the male.

5. *Heritable effects of chemically differing media on the fission rate of Paramecium caudatum.* A. R. MIDDLETON, University of Louisville.

Can we change the living system by chemical means so that the change will persist in to later generations? To attack this question the organism employed was *Paramecium caudatum* and the character considered is the highly modifiable but heritable fission-rate. For one hundred and thirty days two sets of twenty lines each comprising the two halves of a single clone of *Paramecium caudatum* were kept, one set in 1/32 per cent Horlick's Malted Milk prepared with distilled water and the other in 1/32 per cent Horlick's Malted Milk prepared with 2/10 per cent normal saline. After ten, twenty, thirty, forty, and eighty days' exposure to these chemically diverse environments the two sets were duplicated and kept for ten, twenty, sixty, ninety and fifty days, respectively, in 1/10 per cent normal saline malted milk. During the thirteen ten-day periods of the main experiment the saline lines divided characteristically more rapidly than the distilled-water lines. This relatively faster rate of division of the saline set persisted during ten days' cultivation in intermediate saline subsequent to ten days in extremes, during twenty days in intermediate saline subsequent to twenty days in extremes, and during sixty days in intermediate saline subsequent to thirty days in extremes. An exposure of forty days or longer to extremes produced the reverse effect when the duplicate sets were cultivated in intermediate saline. This result may be due to some undetectable injury resulting from long-continued exposure to relatively high salinity.

6. *Heredity of resistance to tuberculosis in guinea-pigs.* (Lantern.) SEWALL WRIGHT and PAUL A. LEWIS, Bureau of Animal Industry, and Phipps Institute.

The resistance to tuberculosis has been tested in over eleven hundred guinea-pigs belonging to five closely inbred families, the crosses between these families and a random bred control stock. It has been found that sex, and even three-fold differences in age, rate of gain, and weight have only a slight effect on length of life after inoculation. In striking contrast to these negative results, are the great differences among the inbred families, differences which are not correlated with the differences in fertility, weight and vitality. The progeny of crosses are in general at least equal to the better of the two parental families. Resistance

is thus dominant over susceptibility. There is equal transmission by sire and dam and to sons and daughters. In particular crosses, the average of the progeny is consistently superior to either parental line, indicating that the latter are susceptible for different reasons, each being able to supply a dominant resistance factor lacking in the other. In the whole crossbred stock, over 30 per cent of the observed variation is determined by the amount of blood of the best inbred family, as compared with less than 10 per cent due to age, weight and rate of gain combined, and leaving about 60 per cent due to conditions at or following inoculation.

7. *Data on the inheritance of spurs in female fowl.* H. D. GOODALE, Massachusetts Agricultural Experiment Station.

By selection, a race of fowls having spurred females has been produced. Two of these were crossed with a race (Cornish) in which the females are never spurred.  $F_1$  is spurless. Segregation occurred in  $F_2$ , the ratios indicating more than one set of mendelian factors.

8. *Heredity of build.* (Lantern.) CHARLES B. DAVENPORT, Station for Experimental Evolution, Cold Spring Harbor.

A consideration of the best quantitative expression of build; the environmental and general physiological factors influencing build. The hereditary factors determining build and their method of action, together with some discussion of the interdependence of the general physiological and hereditary factors.

9. *Effective reverse selection in bar eye of *Drosophila* due to the appearance of mutations.* (Lantern.) CHARLES ZELENY, University of Illinois.

Reverse selection started in the 34th generation of the high line of the white-bar series was continued for seven generations. An effective decrease in mean facet number was obtained in the first two generations of both females and males and in the seventh generation of the females. The results are due to the appearance of two mutations, one, the ultra-bar, which has been observed on several other occasions and the other a factor which causes a return of the mean to that of the unselected population. While the prevalence of lethal factors in the direct line of the selection stock suggests that the effectiveness of reverse selection may be due to their presence, all the evidence cannot be brought into agreement with such a view.

10. *Genetic mosaics and ontogenetic abnormalities in the parasitic wasp, *Hadrobracon*.* (Charts.) P. W. WHITING, University of Iowa.

Genetic differences are orange eye-color, completely recessive to black and defective venation, irregularly recessive to normal. Normal inheritance is sex-linkoid. Sexes are readily distinguished by antennae, abdominal sclerites, and external genitalia. Patroclinous and mosaic males have all external characters and usually gross internal anatomy normal for male. They are regularly produced in variable percentage from cross of black male by orange female. Much more rarely they arise from black female by orange male, but these have thus far been patroclinous for wing character only. They are usually sterile, but if fertile transmit either paternal or maternal characters. In one case both were transmitted and male bred like a zygote. A single sterile male with orange eyes and black ocelli arose from heterozygous female crossed to black male. Only two

gynandromorphs have been found. One was female except for one-half of the abdomen which was clearly male. The other had anterior half male, posterior female. Mosaic males may be due to failure of nuclear fusion at fertilization. Gynandromorphs arise from mixture of haploid and diploid tissue. The male which bred like a zygote may be tentatively explained as a sort of gynandromorph. Certain variations affecting external genitalia produce what was at first thought to be 'intersexes.' These have now been shown to be accidents of growth not correlated with sex.

*11. Olive, a mutation in Colias philodice.* JOHN H. GEROULD, Dartmouth College.

Olive-green caterpillar color is recessive to normal grass-green. The eye of the butterfly developed from an olive-green caterpillar is not normal yellow-green, but olive-green. That the recessive gene probably adds to the normal grass-green hemolymph an orange pigment not yet detected in the blood itself, or at least a determiner of such pigment, is demonstrated by the fact that certain wing scales of the mutant butterfly always show a peculiar orange or buff pigmentation, not normal in this species. This orange pigmentation is most marked on the under surface of the hind wings and of the tip of the fore wings, parts most directly exposed during wing development in the pupa to the action of the hemolymph. The orange-producing gene for 'olive,' acting through the blood upon the grass-green pigment derived from chlorophyll and passed on by the blood to the hypodermis of skin (larva) and eye (butterfly), turns this pigment olive-green; the altered hemolymph, acting upon the wing scales, turns them orange. This gene interacts with another recessive factor, viz., that for blue-green blood and caterpillar color, to give a 9:3:4 ratio (9 grass-green, 3 olive-green, 4 blue-green). The delicate purple sheen that appears in the skin of certain blue-green caterpillars (with blue-green blood) probably indicates the presence and action in them of the recessive olive factor. 'Olive'  $\times$  'olive' breeds true, but certain individuals heterozygous for blue-green produce 25 per cent of blue-green caterpillars.

*12. Incomplete synapsis of chromosomes and its possible relation to linkage variations.* (Lantern.) C. W. METZ, Carnegie Institution of Washington.

In flies of the genus *Dasyllis* certain chromosome pairs appear to undergo incomplete synapsis during spermatogenesis, with the result that certain parts of the chromosomes remain well separated throughout the spermatocyte growth period. The process is uniform and constant in these particular chromosomes, which can be identified by their size and shape. Such chromosome behavior recalls the genetic behavior of the low crossover strains of *Drosophila melanogaster* (Sturtevant, Detlefsen) in which crossing over is greatly reduced or eliminated in certain 'regions' of particular chromosomes. One simple assumption that might account for this result is that of incomplete synapsis in the chromosomes involved. Unfortunately, *Drosophila* is not favorable for a cytological study of this question and *Dasyllis* is unsuitable for genetic analysis, so that the two lines of evidence cannot, as yet, be obtained in the same animal. However, the data from *Dasyllis* leave little doubt on one of the main points, namely, that synapsis is not necessarily uniform but that different parts of a chromosome may differ radically in this respect. This fact lends support also to the hypothesis that the grouping or clumping of genes in the chromosome maps

of *Drosophila* may be due to differences in the intimacy of synapsis in the corresponding regions of the chromosomes.

13. *A cross in guinea-pigs best explained by assuming 75 per cent crossing over.* (Lantern.) HEMAN L. IBSEN, Kansas State Agricultural College.

The factors involved are *P*, dark-eyed, *p*, pink-eyed; *Cr*, non-yellow, *Ca*, albino; *e<sup>p</sup>*, tortoise, partial extension of black, *e*, non-extension of black. Two crosses were made: 1) A pink-eyed self-white, *pp ee Cr Cr* was crossed with a 'dark-eyed' albino, *PP ee Ca Ca*, and the dark-eyed self white offspring, *PP ee Cr Ca*, were inbred, producing 120 dark-eyed self whites and 64 which were either albinos or pink-eyed self whites. 2) A pink-eyed self white, *pp ee Cr Cr* was crossed with a 'dark-eyed tortoise' albino, *PP e<sup>p</sup> e<sup>p</sup> Ca Ca* and the dark-eyed non-yellow tortoises, *Pp<sup>e</sup> e Cr Ca*, were inbred, producing 51 dark-eyed non-yellow tortoises, 6 pink-eyed non-yellow tortoises (*pp e<sup>p</sup> Cr*), 16 dark-eyed self whites (*P ee Cr*) and 38 pink-eyed self whites or albinos.

The first cross was made to determine whether a 9:7 ratio would be obtained in the  $F_2$ , and the second, whether a 27:8:9:19 ratio would be obtained. Neither was closely approximated. However, by assuming that *P* and *Cr* were linked, and that 75 per cent crossing over took place in both sexes, it was found that the theoretical ratio (41 dark-eyed : 23 pink-eyed or albinos) fairly closely approached the observed ratio. If in addition one assumed that *e<sup>p</sup>* was not linked to the other two factors, the theoretical ratio for the second cross (123 dark-eyed non-yellow tortoises : 41 dark-eyed self whites : 21 pink-eyed tortoises : 71 pink-eyed whites or albinos) was also found to be fairly close to the observed ratio. This hypothesis is tentative. Final proof can be obtained only by mating the heterozygotes back to recessives.

14. *A linkage diagram of nine factors for color patterns in Apotettix eurycephalus Hancock.* ROBERT K. NABOURS, Kansas Agricultural Experiment Station.

The recessive, gray normal (primitive, or wild type) and nine dominant color characters, all from nature, are as follows:  $+/+$  = grey normal; *M/M* = V-pattern; *Y/Y* = white-spot; *O/O* = white-all-over; *R/R* = yellow-all-over; *W/W* = yellow-striped-femora; *Z/Z* = brown-tipped-femora; *G/G* = brown-stripe; *K/K* = white-stripe; *T/T* = red-all-over (American Naturalist, 1919). During the ten years of the experiment, breeding bisexually and parthenogenetically, these factors have been paired 200,942 times in the females, ranging from 15,142 pairings of *R* and *K* to 1,794 of *O* and *W* (crossing over occurs only to a negligible extent in males). *M* and *Y* are nearly, if not exactly, allelomorphs. *O*, *R*, *W* and *Z*, forming a group of multiple allelomorphs, average 5.82 per cent of crossing over with *M* and *Y*, *G*, *K* and *T*, each allelomorphic to the other, average 0.63 per cent of crossing over with the *O*, *R*, *W*, *Z* group. The average direct crossing over between *M* and *Y* and the *G*, *K*, *T* group amounts to 6.12 per cent. The difference between this longer distance and the sum of the two shorter, amounting to 0.33 per cent may partly be accounted for by double crossing over, which infrequently occurs. There are considerable divergences among several pairs in the crossing over percentages, and wide divergences among given pairs between the percentage of linkage, or coming together, and the percentage of separating, or crossing out.

15. *Genetic analysis of low crossover stock, produced by selection.* J. A. DETLEFSEN and L. S. CLEMENTE, University of Illinois.

A stock of *Drosophila melanogaster* produced by selection and giving about 5 to 6 per cent crossing over between red eye and long wing vs. white eye and miniature wing was mated to a stock of white eye, crossveinless, cut-wing, miniature, forked. The results of the  $F_1$  and  $F_2$  show that: 1) One of the two homologous chromosomes alone has been affected, namely, the one carrying red-eye and long-wing. The other chromosome carrying white-eye and miniature-wing was apparently unaffected by selection. 2) The regions from red to cross-veined and from cross-veined to not-cut are about 13 and 7 units long, respectively, but in our low selected series these regions have been very greatly reduced, perhaps to less than one unit. The region from not-cut to long-wing is about 16 units normally, but has been reduced to about one-fourth of this value. The region to the right of long-wing up to not-forked has been slightly reduced in crossover value, although this region was not involved directly in the process of selection. The total distance between red-eye and long-wing is about 33 units normally but in our low series it has been reduced to 5 units, or at least the percentage of crossing-over is about 5 per cent. Comparison of  $F_1$  and  $F_2$  distributions show that the latter are more variable.

16. *Ten years with the self-fertilized line of *Lymnaea columella*, Say.* HAROLD SELLERS COLTON, Zoological Laboratory, University of Pennsylvania.

On the night of February 4, 1911, a pond snail from a tank in the vivarium of the University of Pennsylvania laid a mass of eggs. The young snails hatching from those eggs were isolated from one another and so prevented from mating. Although cross breeding was prevented, yet for forty-seven generations the line has continued to live because the snails self-fertilize their own eggs. Extending over a period of almost ten years inbreeding of the very closest sort has been practiced, resulting in an animal of great theoretical gametic purity; greater perhaps than any other animal which reproduces sexually. This line of snails agrees with the experience of King, Wright, and others, namely, that inbreeding does not appreciably affect the viability of the race.

17. *Results of eight years of inbreeding of Rhode Island Red fowls.* (Lantern.) L. J. COLE and J. G. HALPIN, University of Wisconsin.

Selection on the basis of a non-vital character (plumage color) resulted in a rapid deterioration of the stock, which ran out completely in four years. Egg production was affected to some extent, but the deterioration was particularly noticeable in vitality, as measured by hatchability of the eggs. In a second phase of the experiment the inbreeding has been of the same intensity, but selection has been based on vigor, as measured by good hatchability and strong vigorous chicks. This has resulted so far in raising the general vitality of the stock, though there appears to have been a loss in egg production, which has not been considered directly in the selections. The results can be interpreted as due to the inheritance of lethal, semilethal or physiological-defective factors, though no such have as yet been experimentally isolated.

18. *Inheritance of color in the domestic turkey.* (Continued.) W. R. B. ROBERTSON, University of Kansas.

In 1920 a black male was bred to a bronze female. The  $F_1$  generation was black except for a few barred feathers among the coverts of the tail. Also a narragansett male was bred to a bourbon red female. The  $F_1$ 's were much like the narragansett, but showed distinctly the influence of the red in increased amount of the red or auburn in the feathers. In 1921 an  $F_1$  black (bronze) male was mated to three  $F_1$  Narragansett-red females. In  $F_2$  there were 4 black males and 2 black females (all showing a very few barred-pattern feathers among the tail coverts), 2 rusty black males showing more traces of barring than in the black, 1 bronze male and 6 bronze females, and 4 bronze-red males and 2 bronze-red females. The classes theoretically should be black (narragansett), black-red, bronze (narragansett), and bronze-red. All classes are represented. The small numbers may account for the absence of females in the black-red. The experiment shows that the narragansett pattern is an additional allelomorph. The series is black, bronze, narragansett, and bourbon red. This order also indicates their relative dominance, black being most dominant, bronze next, etc. Bronze seems to be completely dominant to narragansett.

19. *Orthogenesis of non-homochromic pigmentation in chromodorids.* W. J. CROZIER, Rutgers College.

In all species of *Chromodoris* for which color descriptions exist, indication is had of the presence of two distinct types of integumentary pigments. One of these substances has a characteristic band absorption spectrum and other properties permitting ready identification. As obtained from different species (Bermuda, California), this substance is found to be chemically very similar, perhaps identical. Since the habits of the various species are different, the relation of coloration to habit may be interpreted in accord with modern genetic views of adaptation.

20. *Breeding experiments with the viviparous teleosts, Xiphophorus helleri and Platypoecilus maculatus (Günth.).* A. W. BELLAMY, University of Chicago.

The material is admirable for studies pertaining to heredity of physiological and morphological characters; sex-differentiation; and experimental evolution. *X. helleri* is conspicuously marked by a lateral red stripe and other minor color variations. Dorsal fin has  $13 \pm 1$  rays. A ventral prolongation of the caudal ('sword-tail') is characteristic of males. Form-index—body length/greatest depth—3.5 for males; 3.0 for females. *P. maculatus* presents four color varieties: 'rubra,' 'nigra,' 'pulchra,' (spotted), 'plain.' Dorsal fin has  $10 \pm 1$  rays. Form-index for males and females approximates 2.7. In males of both genera the anal fin becomes modified (*X. helleri*,  $136 \pm 60$  days; *P. maculatus*,  $141 \pm 80$  days after birth) to form an intromittent organ. Sex ratios are very erratic, especially in *X. helleri* which averaged (317 fish) 100 males to 67.7 females; *P. maculatus*, (669 fish) 100 males to 147.7 females.

Most of the possible straight, reciprocal, and back-crosses have been made. Only general results from the cross: 'nigra' male and *X. helleri* female can be given here.  $F_1$  hybrids are uniform and deep greenish black much darker than darkest parent. (True of most black hybrids.) One mating gave 8 males, 1

female; another 0 males, 10 females; another 6 males, 0 females. Dorsal fin rays, 11-12. Males have small 'sword'—a male secondary sex character inherited through the female, as Gerschler showed.  $F_2$ : one mating gave variable greenish blacks, males 12; females 30; variable whites, males 30, females 5. Two blacks approximated the 'nigra.' Several white males show the 'sword.' A peculiar type of sex-linked inheritance is indicated in this and other crosses. Most  $F_1$  hybrids are fertile; many  $F_2$  and sesqui-hybrids are sterile. Intersexes occur. Form indices vary from one extreme to the other in both sexes.

21. *The elimination of the sex-chromosome by x-rays: A modification of the germ plasm produced by an external agent.* JAMES W. MAJOR, Union College.

Homozygous, wild type, virgin female *Drosophila* were treated with x-rays and mated to white-eyed males. None of nineteen control pairs produced any white-eyed flies, although they produced over 6500 offspring. Twelve out of fifteen treated females produced white-eyed males.

22. *Hybrid vigor, hybrid weakness, and the chromosome mechanism of heredity. An experimental analysis of the physiology of heredity in the reciprocal crosses between two closely associated species of sea-urchins, Strongylocentrotus purpuratus and S. Franciscanus.* H. H. NEWMAN, University of Chicago.

Much has been written of late about hybrid vigor, in interspecific  $F_1$  hybrids, especially among plants. East and Jones have attempted to explain the added vigor of such hybrids as due to the summation in the hybrid of the dominant or favorable characters of the two species. They imply that all  $F_1$  interspecific hybrids exhibit hybrid vigor. The writer has for a long time called attention to the fact that, in every lot of cross-fertilized eggs, weak and defective individuals are as obvious as are those that show hybrid vigor. In 1908 the writer explained the extra vigor of the surviving *Fundulus* hybrids as the result of the chance combination of the more favorable or dominant specific characters, and the relative lack of vigor of other individuals as the result of less favorable combinations of specific characters.

In Echinoderm hybrids, as in Teleost hybrids, the entire history of large groups of individuals can be followed from fertilization to a definitive larval condition. In the cross, *Strongylocentrotus purpuratus* ♀ × *S. franciscanus* ♂ there is frequently very pronounced hybrid vigor associated with extreme hybridity in many positive larval characters; yet there is also equally prevalent hybrid weakness. In the reciprocal cross there is no hybrid vigor, but only hybrid weakness, and very little, if any, paternal heredity. These facts form the basis of certain theories of heredity that cannot be put forth in an abstract. It is, however, the writer's belief that they are compatible with current theories of the chromosomal mechanism of heredity.

23. *Changes in egg production at the Massachusetts Agricultural Experiment Station.*

H. D. GOODALE, Massachusetts Agricultural Experiment Station.

A history of the steps taken to date. Egg production has been increased by selection, based on the consideration that at least five major components can be recognized in one egg record, namely, maturity, winter pause, broodiness, intensity, and date of last egg. Thus far, attention has centered on decreasing the average age at which the first egg is laid, the decrease being fifty-eight days, with

corresponding increase in winter production. Broodiness has also been reduced. The mean annual production has been increased from 121 to 185 eggs.

24. *Variations in the number of vertebrae and other segmental characters of fishes correlated with the temperature of the water during development.* CARL L. HUBBS (introduced by L. V. Heilbrunn), Museum of Zoology, University of Michigan.

The writer for a number of years has been studying the correlation between altered environmental conditions and the number of vertebrae and other segmentally arranged structures in fishes. Johannes Schmidt has been carrying on a series of intensive investigations which deal with the same problem, and which are for the greater part rather closely paralleled by the writer's studies. Both of us have obtained, independently, a rather large volume of experimental and observational evidence indicating that the meristic characters displayed by an individual fish are determined not alone by heredity, but in part also by the environmental conditions, notably temperature, which prevail during some sensitive developmental period. We have further demonstrated that altered environmental conditions induce differences highly similar to those which characterize local races in fishes, some of which have been proved genetically distinct. For example, differences induced by cold are in the same direction as those characterizing races inhabiting cold water. The writer is planning a series of investigations by which he hopes to arrive at an explanation of these phenomena.

## B. EMBRYOLOGY

1. *Cell behavior in tissue cultures.* (Lantern.) H. B. GOODRICH, Wesleyan University.

1. A non-amoeboid type of cell motion.—A study has been made of a peculiar mode of motion of certain isolated cells observed in tissue cultures of *Fundulus heteroclitus* and *F. majalis*. These cells are identical with those observed by Doctor Dederer (paper in press) and identified as mesenchyme cells. These cells are described as having peculiar fan-like expansions by which they are anchored to the cover-glass. The present observations indicate that these fans form the motor organs of the cell. The motion is apparently not amoeboid. The fan seldom changes in form while in motion and it has the consistency of a colloid gel. It is a delicate membrane which glides on the under surface of the coverslip and pulls after it the body of the cell. The maximum observed rate of motion was 6 $\mu$  per minute.

2. Tactile reactions. Experiments utilizing the micro-dissection needle show that the cell body may be touched by the needle and thus be stimulated to release the fan and to contract. The tactile reactions of the pigment cells have also been studied. The black melanophores, red chromatophores, and yellow chromatophores form a series, in order named, of increasing tactile response (as shown by withdrawal of pseudopodia) to the touch of the micro-dissection needle.

2. *Experiments with Necturus and axolotl thyroids.* W. W. SWINGLE, Yale University.

1. Adult *Necturus* were fed large quantities of physiologically active thyroid, anterior lobe pituitary and simultaneously transplanted with frog thyroids. Results negative at the end of four months despite the enormous thyroid dosage..



2. Thyroids of untreated *Necturus* were transplanted into immature *R. clamata* larvae without limbs. Ten to fourteen days later the larvae presented the symptoms of hyperthyroidism, such as fore and hind leg development and practically complete tail atrophy. Despite its larval characters *Necturus* possesses highly active thyroid glands and seems to have lost the ability to transform, under stimulus of thyroid feeding.

3. The thyroids of an axolotl 14.25 inches long and at least four years of age were transplanted into *R. clamata* larvae without limbs. The glands were large, vascular, and the vesicles distended with a fluid, presumably colloid. The glands were cut into six parts and six larvae grafted. One tadpole died two days later, the remainder showed the typical hyperthyroid reaction upon the eighth day. Two weeks from the date of grafting the larvae had fore and hind legs, and showed marked tail resorption. Thus a single axolotl thyroid when extirpated contains enough active hormone practically to metamorphose five tadpoles, but when left unmolested within the axolotl's body is incapable of initiating metamorphosis. Axolotl neoteny is apparently due to the inability of the thyroid gland to excrete into the blood stream its fully formed and physiologically active hormone. A releasing factor is lacking. Axolotls readily metamorphose when fed thyroid in large quantities. The pituitary of axolotl appeared normal; when transplanted, the gland was resorbed and results negative.

3. *Chorionic fusion and augmented twinning in the human tube.* LESLIE B. AREY, Northwestern University Medical School.

The data from all known cases of human tubal twins indicate that the ratio of monochorial to dichorial specimens is about fifteen times greater in the tube than in the uterus. Illustrative stages are at hand which indicate that this disproportion results in part from the secondary fusion of the chorionic sacs of dizygotic individuals. Hence for man the term 'monochorial' need not always connote a single ovum origin. However, a still more potent factor appears to be an actual augmentation of the twinning impulse. Its cause is most clearly referable to tubal inflammation and its sequelae. The same delays and arrests which cause and follow tubal implantation are largely responsible for both the increased twinning and the excessive malformation of tubal specimens.

4. *The influence of thyroid-gland feeding upon tadpoles from which the thyroid gland and the buccal anlage of the hypophysis have been removed.* BENNETT M. ALLEN, University of Kansas.

These experiments were performed upon *Rana sphenoccephala* 1) normal controls; 2) controls cut as for hypophysis removal; 3) thyroidless tadpoles; 4) those deprived of the buccal anlage of the hypophysis, and 5) tadpoles deprived of both thyroid and buccal anlage were fed weighed quantities of sheep-thyroid preparations. The purpose was to determine whether the influence of thyroid feeding would be the same in each case. Tadpoles in young feeding stages were employed in lots of twenty-five. It appeared that the presence or absence of these glands in the tadpoles had little control upon the influence exerted by thyroid feeding upon limb development. The tail was not so quickly reduced as a result of administration of thyroid to groups 4 and 5 as in groups 1, 2, and 3. It is possible that future experiments in administering more diluted doses of thyroid prepara-

tions may prove a more delicate test. A study of the thyroid glands of controls and of tadpoles deprived of the buccal anlage of the hypophysis fails to show any modification of gland development or of colloid accumulation to result from thyroid feeding.

### C. CYTOLOGY

1. *A determination of the degree of constancy in the nuclei of certain organs in Hydatina senta.* H. J. VAN CLEAVE, University of Illinois.

Eric Martini ('12) maintained that each individual of *Hydatina senta* bears a fixed total number of 959 nuclei for the entire organism. A. F. Shull ('18), using serial sections, studied the vitellaria and gastric glands of this species to determine a measure of the exactitude of nuclear constancy. In 245 vitellaria he found 4 per cent which did not contain the customary eight nuclei. Over 7 per cent of the gastric glands examined by him displayed numbers of nuclei other than the characteristic six. Age, culture media, and male and female determining strains were considered, but there was no striking difference in the numbers of aberrant glands under different conditions of his observations. Subnormal numbers of nuclei were more frequently encountered than supernumerary and odd numbers were more frequent than even in the variants described by Shull.

An investigation of stained whole mounts, begun by the present writer soon after the appearance of Martini's monograph, has just been completed; 435 gastric glands were examined without discovering a single variant from the customary six nuclei in each. In 770 vitellaria, three were found with supernumerary nuclei, two with ten each and the third with twelve nuclei. Not a single subnormal number was observed nor were there any variants bearing odd numbers. Frequently vitellaria were observed with the number of nuclei normal but with unusual appearance, strongly simulating conditions in amitosis. Every aberrant individual encountered in this study presents evidences of an inconsistency superimposed upon an absolute constancy in nuclear numbers.

2. *Further studies in the cytology of Anisolabis maritima, Bon.* (Lantern.) S. I. KORNHAUSER, Denison University.

It was previously determined that the diploid chromosome number is 25 in the male and 26 in the female. Eleven tetrads and a hexad appear in the primary spermatocyte metaphase plates, whereas in the second spermatocytes half show 12 and half 13 dyads. A detailed study of the hexad was made. It was found to be an XXY complex made up of three distinct spermatogonial chromosomes. Unlike the 22 autosomes these three remain compact during the leptonema. The two X-chromosomes soon come to lie side by side and while the autosomes are conjugating the Y-chromosome approaches and is in contact with the two X-elements by a narrow strand. Parasyndesis of the autosomes being completed the Y-element now separates and remains apart from the XX-element during the zygonema and pachynema. The X-elements stain like chromatin, but the Y-element is lighter in chromatin stains, is vacuolated and after Benda fixation takes mitochondrial stains.

With the onset of the strepsinema XX and Y-elements fuse, and soon give off a spherule which stains like mitochondria and which gradually diminishes in size and disappears before the first meiotic spindle is formed. The XXY-hexad

comes out of the fused mass. From now on the Y-element reacts to stains exactly like chromatin. In the first meiotic division 11 autosomal dyads and the XX-element pass to one pole whereas 11 autosomal dyads and the Y-element pass to the opposite pole. During the interkinesis the two components of the XX-element separate so that half the second meiotic division plates show 13 dyads.

3. *The occurrence of telosynapsis in the male germ cells of Leptocoris trivitattus*, Say. (Hemiptera). HARRY B. YOCOM, University of Oregon.

In *Leptocoris trivitattus*, Say, the chromosome complex of the spermatogonium consists of thirteen chromosomes, ten autosomes, two 'M' chromosomes, and one accessory chromosome. After the last spermatogonial division all of the chromosomes except the accessory enter into a fine spireme which is tightly tangled toward the center of the nucleus. As growth of the primary spermatocyte proceeds this thread becomes thicker and unwinds, so that it fills the nucleus with a loosely convoluted thread. Near the end of the growth period this thread again contracts into a mass concentrated toward the center of the nucleus. There is no indication of any polarization or pairing of the thread during this period of synizesis. Later this spireme spreads out until it fills the nucleus with a lightly staining very diffuse work, the organization of which it was impossible to determine. The chromatin of this diffuse mass becomes condensed into a number of masses, probably twelve, which as they become compact unite end to end to form the bivalent chromosomes of the primary spermatocyte. Conjugation of the homologous chromosomes is therefore by telosynapsis. The accessory chromosome can be distinguished at all times during the growth of the spermatocyte by its compactness and rounded contour. At the first spermatocyte division which separates the homologous chromosomes, the accessory chromosome divides equally. At the second maturation division the accessory passes to one pole undivided.

4. *Studies on the cells of sheep with special reference to spermatogenesis, oögenesis, and sex-determination*. (Lantern.) J. E. WODSEDALEK, University of Idaho.

Thirty-three chromosomes occur in the spermatogonia. One, the sex-chromosome, is distinctly larger than the others. Seventeen chromosomes appear in the primary spermatocyte division, of which sixteen are bivalent and the other is the unpaired sex-chromosome. This division gives rise to two types of secondary spermatocytes since the sex-chromosome passes to one pole undivided. Finally two types of spermatids are produced. All of the spermatids contain the sixteen autosomes while only half of them possess the additional sex-chromosome. Thirty-four chromosomes occur in the oögonia; two of these are the sex-chromosomes. Seventeen bivalent chromosomes appear in the primary oöcyte division; one of these, clearly the largest, is the bivalent sex-chromosome. The reduced number of chromosomes in the female is sixteen autosomes and one sex-chromosome, the same number being thrown off with the first polar body. This takes place in the ovary while the oöcyte lies in the graafian follicle. Various stages of this process have been observed in a number of cases. The prevailing number of chromosomes in the somatic cells of male embryos and fetuses corresponds to the number present in the spermatogonia. While the number in the somatic cells of the female corresponds to the number present in the oögonia.

5. *The structure and division of Trichomonas augusta alezeieff.* D. H. WENRICH, University of Pennsylvania.

This flagellate, found in the rectum of many Amphibia, possesses the organelles characteristic of the genus: nucleus; cytostome; and the blepharoplast to which are attached a group consisting of three anterior free flagella, a posterior flagellum which is also the chromatic margin of the undulating membrane, the chromatic basal rod, the parabasal body and the axostyle. As in *Trichomonas muris*, the parabasal body appears only after certain fixatives such as weak Flemming's. The undulating membrane contains near the chromatic margin a secondary filament of small caliber. This is well shown after fixation with Bouin's fluid. During the prophase of division the nuclear chromatin gradually organizes into double (split?) chromosomes and a small new chromatic basal rod appears, attached to the blepharoplast. Later this rod is seen to be accompanied by a small new undulating membrane. The blepharoplast divides, the daughters separating but remaining connected by the chromatic parademesome. In the metaphase plate seven double (split?) chromosomes can be counted which at first have their long axes perpendicular to the spindle axis but during separation become elongated parallel to the spindle axis. The axostyle becomes detached from the blepharoplast and gradually disintegrates. During the telophase the daughter nuclei become organized and a new axostyle grows out from each daughter blepharoplast. Excepting the blepharoplasts and nuclei, the new organelles needed to complete two sets appear to develop anew. When two complete sets of organelles have developed, the cell body divides.

6. *Multinucleated germ cells in the Columbian ground-squirrel.* (Lantern.) J. E. WODSEDALEK, University of Idaho.

Cells containing two or four nuclei were rarely found by the writer in his studies on the male germ cells of swine, horses, cattle, and sheep, and a few cells with eight nuclei were observed in the cat. Multinucleated cells are much more numerous in the testes of the Columbian ground-squirrel (*Citellus columbianus columbianus*). Cells with two, four, and eight nuclei were occasionally found in the tissue taken from eighteen individuals. In the tissue taken from two individuals not only are the cells containing two, four, and eight nuclei plentiful, but cells with as many as sixteen and thirty-two nuclei, of the spermatid type, are fairly common. The two males concerned were vigorous specimens and their germ cells were extremely active. Numerous mature spermatozoa and others in the process of development were present. Other normal cells in the various stages of mitosis were also abundant in the immediate neighborhood of the multinucleated cells.

7. *Seasonal changes in the testis of Gambusia affinis, the top-minnow.* S. W. GEISER (introduced by S. O. Mast). The Johns Hopkins University.

The testes develop from the typical anlage. During early life, they are separate, except at their posterior ends; at the age of two to three months, they fuse more or less completely along the median line. The testis consists of fine connective-tissue stroma which becomes apparent as investments of the cysts when the testis is in active spermatogenesis. In adults the longitudinal testicular canal (vas deferens of German workers) is tubular, tortuous, usually paired,

but sometimes fused. Testes fluctuate in volume between summer and winter, but the fluctuation is not very marked (eight-fold volume-increase in summer, maximum found). Testes in Winter are filled with spermatozeugmata, which greatly swell the 'vas deferens,' so that it sometimes comprises 62.4 per cent of the volume of the testis, as compared with 1.2 per cent to 1.5 per cent at the high-point of spermatogenesis (May/June.) The testis does not possess cords of sex-cells for the renewal of the testis after spermatogenesis. There are no spermatogenic tubules. The spermatogonia develop apparently from inconspicuous germ-cells lying in the stroma of the testis, between the cysts. These cells migrate peripherally (outside the zone of the vas deferens) and give rise to new spermatogonial cysts. The period of greatest sexual activity (max. testis-volume) in early spermatogenesis is March/July. The onset of this period appears to be largely conditioned by temperature and light conditions. In November/February, the testes attain their minimum size. With the onset of cold weather, sexual activity ceases. No spermatogenesis occurs during the cold weather.

All the cells in a spermatogonial cyst are in approximately the same stage of spermatogenesis. A spermatozeugma may have from 5000 to 13000 spermatozoa.

8. *Changes in the vaginal epithelium of the guinea-pig during the oestrous cycle.*

R. M. SELLE (introduced by J. A. Long).

The oestrous cycle in the guinea-pig shows four clearly defined periods or stages, in addition to the interval, which correspond to similar stages in the rat. In the first stage, which apparently was overlooked by Stockard and Papanicolaou, the vaginal smear contains large, vacuolated, granular epithelial cells only. These epithelial cells are derived from the superficial layers of the mucosa beneath which cornification has been going on. The mucosa is highest at this time. In stage 2 the superficial epithelial cells are gone, leaving uppermost the cornified stratum. The latter by scaling off furnishes the typical, non-nucleated, flake-like cells of the smear. Up to this point the similarities of the rat are close. However, in the guinea-pig the whole cornified layer, not the whole epithelium, with some of the underlying non-cornified epithelial cells may be shed en masse like a cast. Whereas in stage 3 in the rat the granular, cheesy material consists of the elements of the cornified stratum, rapidly desquamated singly or in small groups until the stratum is entirely lost, in the guinea-pig the cheesy mass described by Stockard and Papanicolaou is constituted of the deeper-lying non-cornified epithelial cells which are shed in large numbers. In the last stage (4) leucocytes appear as described by Stockard and Papanicolaou in their stage 3. No state has been found corresponding to their 4. There is no evidence of any important contribution by the uterus to the cellular contents of the vagina, for the latter is practically identical in normal pigs and in hysterectomized pigs.

#### D. COMPARATIVE ANATOMY

1. *Metamorphic changes in the digestive system in Rana pipiens and Amblystoma tigrinum.* ALBERT KUNTZ, St. Louis University School of Medicine.

The period of metamorphosis and the coincident period of fasting occupies approximately ten days in *Rana pipiens* and nine days or less in *Amblystoma tigrinum*. Quantitative data at hand indicates the following changes during metamorphosis.

	R. PAPIENS	A. TIGRINUM
	<i>per cent</i>	<i>per cent</i>
Average reduction in total weight.....	57.3	28.7
Average reduction in length of stomach and intestine.....	82.2	45.8
Average reduction in length of stomach.....	54.5	54.8
Average reduction in weight of stomach and intestine with contents.....	92.8	68.6
Average reduction in weight of liver.....	80	None

The more important histological changes in the stomach and intestine during metamorphosis consist in increasing thickness of the several layers and more compact aggregation of the tissue elements as the reduction in the length of the digestive tube takes place. The increase in the thickness of the several layers does not involve active cell proliferation, but is accomplished by rearrangement and aggregation of the elements already present. These histological findings agree essentially with those of Ratner ('91) in *Rana temporaria*.

2. *The skull of Syngnathus fuscus.* JAMES E. KINDRED, Western Reserve University.

In an earlier paper the primary stages in the development of the skull of *Syngnathus fuscus* were discussed and certain fundamental characters of importance were described and commented upon. That study has been followed by a comparative study of the ossification processes leading to the formation of the adult skull. The material has been described with reference to the modifications which the skull of *Syngnathus* shows when compared with a closely allied form as *Gasterosteus*. In its development, the skull of *Syngnathus fuscus* (pipe-fish) shows certain important differences from the skulls of related forms. The elongate condition characteristic of the skull of the adult fish is arrived at very early in life through the precocious growth of the ethmoid and symplectic cartilages. As a result of this the mandibular apparatus has been carried to the end of the snout and a series of bones developed for the support of the walls of the oral tube. These bones are arranged in such a manner that they form a flexible wall for the tube. They have been homologized with the suborbitals and nasal bones of closely allied species, but in this study I have regarded them as independent elements which are developed in response to local needs and not as modifications of the above bones of other teleosts. Various modifications from ordinary teleostean type of skull have been observed and a detailed description and comparison of the components of the skull has been made and the points wherein this fish is primitive or specialized have been discussed.

3. *The thyroid glands of perennibranchiate amphibians.* W. W. SWINGLE, Yale University.

The thyroid glands of *Necturus* are situated beneath the mylohyoid muscle, at the apex of the triangle formed by the geniohyoid and external cerato-hyoid

muscle. The glands are quite small but are perfectly formed and contain much colloid. In some animals the glands vary in size and location, the vesicles may be few in number but very large. Through the courtesy of Prof. Stejneger of the United States National Museum a specimen of *Typhlomolge rathbuni* was obtained. The animal had been preserved in alcohol for eighteen years. The thyroids could not be located, though vesicular structures filled with fluid and resembling the glands were first mistaken for them. Further investigation of two additional specimens of *Typhlomolge* failed to reveal glandular tissue. The thyroid apparatus of *Proteus* has been described by Franz Leydig, 1853 (*Anatomisch-physiologische Untersuchungen über Fische und Reptilien*, p. 62), and appears not to differ from the condition described for *Necturus*. The thyroids of tadpoles with extraordinarily prolonged larval lives, such as *R. clamata* and *R. catesbeiana* have a meager blood supply compared with adult urodeles such as *Diemyctylus*, *Amblystoma*, and oddly enough, *Axolotl*.

4. *On the muscular system of Ornithodoros moubata*, Murray. E. MELVILLE DU PORTE, Macdonald College, McGill University.

1. A descriptive study of the musculature of this argasid tick. 2. The introduction of a nomenclature similar to that in use by insect anatomists. 3. An attempt to work out the homologies of the muscles.

5. *On the structure and development of a fat body or gland in the rat*. J. A. LONG, University of California.

This body hitherto undescribed for the rat is of considerable size and is lobed or branched. The main portion, somewhat butterfly-shaped in dorsal aspect, lies in a depression of the muscles in the middorsal line between the shoulder-blades. Continuous with the central part or separated from it only by very short spaces are other plate-like parts extending laterally between the layers of muscles. Some run well down between the deep muscles under the scapulae. It is well supplied with blood and is closely associated with vessels going to the body muscles. It is unlike ordinary body fat in color and structure, being brown to salmon colored, and being composed of cells gorged with droplets of lipid of varying size, among which the spherical nucleus is embedded. The lipid is a neutral fat, staining red in Nile-blue-sulphate. This body is first visible in the embryo of seventeen days, after muscle formation has already begun. It increases so rapidly in size that by the end of pregnancy it is not much less in volume than the liver. The general form and arrangement of the various parts is substantially as in the adult. One detached portion extends as far ventrally as the submaxillary salivary gland. It is very highly vascular; each cell has a spherical nucleus and most cells contain one to several lipid globules of variable size; the whole structure reminding one of an organ of internal secretion such as a corpus luteum.

6. *Relation of growth to metamorphosis in the lungless salamander, Eurycea bislineata.* INEZ WHIPPLE WILDER, Smith College.

For uniformity the material used has been taken from one locality. Hatching occurs during late June and early July. Metamorphic individuals are found throughout the year and range from 42 to 76 mm. in length and from 390 to 1085 mg. in weight. Total length frequency graphs based on representative collections at frequent intervals for seven years involving over 2000 individuals show, 1) large variability in different years; 2) an average length of larval life of two years with a range from one to three years; 3) segregation during the first year into a lagging and a precocious group. Experimental study of growth and metamorphosis under laboratory conditions simulating normal food and temperature conditions show, in addition to corroboration of the above conclusions, 1) successive periods of slow and rapid growth; 2) wide variation, in which sex is not the main factor, in growth and metamorphosis of individuals of the same age under the same food and temperature conditions; 3) possibility of prolonging, through the variation of food and temperature, any of the four developmental periods, postembryonic, typical larval, premetamorphic, and metamorphic. Normal tables of total lengths, tail length indices, weights, weight indices, and developmental stages have been constructed based on representative collections approximately uniformly distributed throughout five years. An exact knowledge of the normal range of variation in rate of growth and development of any species seems absolutely necessary as a basis for correct interpretation of the results of any experimental work on the ductless glands.

7. *Degeneration in the pelvis of the female pocket-gopher, Geomys bursarius.* FREDERICK L. HISAW (introduced by J. E. Ackert), Kansas State Agricultural College.

An examination of over three hundred pocket-gophers collected in the vicinity of Manhattan, Kansas, showed that in the mature animals the pelvic bones are completely ossified and meet to form the pubic symphysis. In both males and females the pelvic girdles are greatly reduced in size. This reduction is obviously an adaptation to their fossorial habit, but, as a result, the puboischiatic-vacuity is too small for the birth of young. However, during the breeding season, a degeneration of the pelvic bones of the young female begins in the pubic region, and continues laterally almost to the obturator foramen. After the birth of young, the symphysis is not reformed, and virgins and old females can be distinguished in this way. Females with abnormal reproductive systems, due to abnormal development or to unnatural confinement in cages, retain the closed symphysis. The degeneration in the pelvis seems to be due to internal secretions of the reproductive system, either before or during pregnancy.

## E. ECOLOGY AND ZOOGEOGRAPHY

1. *Chemical composition of some fresh-water organisms.* C. JUDAY, University of Wisconsin and Wisconsin Geological and Natural History Survey.

A number of fresh-water organisms have been subjected to chemical analysis in order to obtain some idea of their food value. The plants included several species of algae and a few of the large aquatics. The animals were represented



by several species of plankton crustacea, crayfish, worms, leeches, insects and insect larvae. Crude protein constituted more than 50 per cent of the dry weight of the plankton algae, but it fell to 10 per cent to 20 per cent in the large aquatic plants. In the animals the crude protein constituted from 36 per cent to 64 per cent of the dry weight of the plankton crustacea and from 35 per cent to 69 per cent in the larger forms, the maximum percentage being noted in the leeches.

The various samples of plant material yielded relatively small percentages of ether extract, only two slightly exceeding 5 per cent, except the diatoms which gave a little more than 13 per cent. The plankton crustacea yielded from 3 per cent to approximately 40 per cent of ether extract, and the larger animals from 3 per cent to 37 per cent. The plant material contained from 2 per cent to more than 10 per cent of pentosans, but all of the animal samples gave less than 2 per cent except a cladoceran, *Holopedium*, which yielded more than 6 per cent. In the plankton algae the ash constituted from 4 per cent to 8 per cent of the dry weight except in the diatoms where it was approximately 40 per cent; the ash amounted to about 25 per cent in the large aquatic plants. In the plankton crustacea the ash varied from about 4 per cent to almost 26 per cent, while in the larger animals it ranged from about 2 per cent in the gyrids to nearly 35 per cent in the crayfish.

2. *Some adaptations of mayfly nymphs to swift streams.* (Lantern.) G. S. DODDS and F. L. HISAW, West Virginia University.

Animals inhabiting standing water have the problem of locomotion in search and pursuit of food and flight from enemies. At will they may rest, floating in the water or resting upon the bottom. In swiftly flowing water there is no opportunity for rest—the problem of retention of position is a continuous one. Many groups of animals have invaded swift streams and successfully met its problems. Fifteen species of mayfly nymphs from ponds and streams in mountains of Colorado well illustrate modes of adaptation to such conditions. The following ways of meeting the difficult problem of retention of position in rapidly flowing water have been observed. 1) Swimming species of fish-like (stream line) form swim well in still water and the stronger swimmers invade the less swift portions of streams. 2) Species of fish-like form of small size have developed strong legs with which they cling to rocks in the swiftest parts of torrential streams. 3) Flattened forms retain position by: a) avoiding direct shock of water; b) flattening of head and legs in such an attitude that the force of the water presses the animal against the substratum; c) development of sucking organs from gills or ventral surface of abdomen; d) development of strong legs; e) avoiding current in crevices.

3. *Some physical factors related to the distribution of littoral invertebrates.* W. C. ALLEE, University of Chicago.

In addition to considering the direct effects of types of bottoms, shores, plants, and currents, this study deals with the possible correlation of salinity, PH, oxygen content and temperature with the distribution of associations of littoral invertebrates in the Woods Hole region. Two series of associations were studied: those of the flats and those of the rocks. In both the salinity regularly increased as one approached open water. Salinity served as a limiting factor particularly in

the associations placed well back on the flats. Next to salinity the pH of the water varies more regularly with the associations than any other factor examined. Temperature and oxygen did not vary so directly. Temperature serves as a limiting factor in low-tide pools and on the exposed flats; free oxygen vanishes from regions with muck bottom and its absence affects distribution in these places. An index figure based on a combination of these four characteristics is found to vary directly with the associations as one passes from open water. Great caution should be used in stressing any one environmental factor in ecological studies. If, on account of lack of time, it becomes necessary to resort to a single environmental factor index in the study of the distribution of animal associations of the seashore, the character of the bottom, the most obvious and longest-used index, is still the least treacherous.

4. *Ecology of parasites of lake fishes.* A. S. PEARSE, University of Wisconsin.

Statistical studies of fish parasites were made in five Wisconsin lakes: Pepin, Geneva, Green, Mendota, Michigan. The following factors appear to increase parasitism in fishes: wide range and variety of habitats, great variety of food, vegetation, shallow water. According to their degree of parasitic infection the fishes studied in two or more lakes rank as follows: dogfish, smallmouth black bass, white bass, rock bass, pumpkinseed, sucker, black bullhead, bluegill, ciscoes, largemouth black bass, pickerel, carp, buffalo, log perch, blunt-nosed minnow, top-minnow, yellow perch, shiner (*Notropis heterodon*), johnny darter, black crappie, shiner (*N. atherinoides*), bream, shiner (*N. hudsonius*).

5. *The eggs of the frogs, tree frogs and toads of Okkefnokee Swamp, Georgia.* A. H. WRIGHT, Cornell University.

In the interior of the Okkefnokee Swamp are eleven species of Salientia, and on its edges are seven other species. Those within are *Rana clamitans*, *R. grylio*, *R. sphenoccephala*, *Bufo quereicus*, *B. terrestris*, *Hyla cinerea*, *H. femoralis*, *H. gratiosa*, *Acris gryllus*, *Pseudacris ocularis*, and *Gastrophryne carolinensis*. Those outside are *Rana aesopus*, *R. catesbeiana*, *R. virgatipes*, *Hyla aquirella*, *H. versicolor*, *Pseudacris* sp., and *Scaphiopus holbrookii*.

The eggs of *R. catesbeiana*, *R. clamitans*, *R. grylio*, *Hyla cinerea*, *H. femoralis*, *H. versicolor*, and *Gastrophryne carolinensis* float on the surface of the water; the eggs of *Hyla gratiosa*, *Acris gryllus*, and *Pseudacris ocularis* are laid singly on the various bottoms of the swamp; the eggs of *Bufo terrestris*, like those of *B. americanus*, are in a single file, not double as in *B. fowleri*, while those of *B. quereicus* are isolated single files of five to seven eggs or with several such files about one focus. The eggs of *Scaphiopus holbrookii* are in bands and those of *R. sphenoccephala* are in a plinth mass like *R. pipiens*. The eggs of *Rana aesopus*, *R. virgatipes*, *Hyla aquirella*, and *Pseudacris* sp. are not sufficiently identified to allow of positive statement. Eggs laid in captivity by pairs captured at night check the identifications of fourteen species. The first species to breed begin in February or March and each species after it has begun continues oviposition after each rainy spell until September or possibly later.

6. *Further studies on the circulation of water in the Bay of Fundy and the Gulf of Maine.* JAMES W. MAYOR, Union College.

Further studies with drift bottles and hydrographic measurements have confirmed the circulation of water as previously worked out.

7. *The effect of temperature in limiting the geographical range of invertebrates of the Woods Hole littoral.* W. C. ALLEE, University of Chicago.

An analysis of the geographical distribution of 241 species taken in collecting operations in the Woods Hole littoral in the last nine summers shows that 55 per cent are south-ranging and 30 per cent are north-ranging species. 40 per cent of the species are not recorded from north of Cape Cod, while 11 per cent are unknown south of the Woods Hole region. This means that the Woods Hole region is located near the northern end of a south-ranging fauna. The winter temperatures of the last four years display marked variation. Those of 1917-18 and 1919-20 were unusually severe, while the alternate years of 1918-19 and 1920-21 were unusually mild. The effect of this variation in winter temperatures on animal distribution should be most marked near the limits of distribution. The unusually cold winter of 1917-18 caused 38 per cent of all the south-ranging species to be present in noticeably fewer numbers the following summer. Of the south-ranging species whose recorded northern limit is Cape Cod, 48 per cent were affected. The similarly cold winter of 1919-20 was followed by an increase of north-ranging forms. The southern extension of north-ranging species is limited by the high summer temperatures of the flats, just as in shallow water the extreme winter cold limits the northward extension of south-ranging species.

8. *Hydrogen-ion concentration of Chesapeake Bay water.* R. P. COWLES and A. M. SCHWITALLA, S. J. (introduced by S. O. Mast), The Johns Hopkins University.

During the early part of June, 1921, while engaged in a biological and hydrographic survey of Chesapeake Bay for the U. S. Bureau of Fisheries, an attempt was made to determine the hydrogen-ion concentration of the bay water in order to see if it bore any constant relation to the distribution of plankton and living organisms in general. One of the stations selected was a 'deep hole' (40.2 meters) a short distance north of the mouth of the Potomac River, where water samples were taken at one and a half hour intervals for twenty-four hours. By colorimetric methods it was found that there was a decrease in the pH value passing from the surface downward; in other words, that the water became more acid. In general a diurnal change in the pH values at all depths was evident; these values as a rule decreasing during the later part of the night and the early morning when chlorophyll-bearing plants no longer carry on photosynthesis. These observations support the results of McClendon for the sea-water at Tortugas and are confirmed in a general way by observations at another twenty-four hour station. Tidal changes were recorded by means of a current meter at all depths almost simultaneously with the pH observations and there seemed to be a tendency towards a rise in pH value when the tides turned in the daytime.

9. *Canuella canadensis* and other Canadian copepod records. A. WILLEY (introduced by A. G. Huntsman), McGill University.

The paper contains the results of the author's observations on the subjects indicated during a number of years, including notes on distribution and ecology.

## F. GENERAL ZOÖLOGY

1. *Note on the rate of growth of teredo and xylotrya.* (Lantern.) THURLOW C. NELSON, Rutgers College and the New Jersey State Board of Shellfisheries.

A platform of dry cypress slats covered with oyster shells was submerged in Barnegat Bay, July 3, 1921, was removed to tidal creek July 25th, and on August 15th collapsed owing to heavy invasion of *Teredo navalis* and *Xylotrya fimbriata*. Slats 2×1 inch contained in cross-section 1 cm. wide, 77 *Teredo* and 3 *Xylotrya*. Average size of *Teredo*: length 15.5 mm., width of body, 2 mm., width at shells, 2.5 mm. Average for *Xylotrya*: length 29 mm., width of body 3.2 mm., width at shells, 4 mm. Maximum number of segments in pallets 14; minimum 5. Upper Barnegat Bay is rarely troubled with 'shipworms.' Abnormal salinity of water resulting from reduced rainfall; water of high salinity creeping up the bay along bottom. Densities of 10082 at surface with 10170 at bottom, 6 ft., were observed. Average density at bottom by platform in bay; 10145, maximum 10170, minimum 10125. Average temperature 25.4°C., maximum 26.6°C., minimum 24.5°C. Average density at surface during same period; 10111, maximum 10117, minimum 10105. During period in creek maximum density 10125, minimum 1009; maximum temperature 30.5°C., minimum 21°C. Ripe eggs present in both species, growth and development must have occurred in less than six weeks.

2. *Observations on sex in the top-minnow, Gambusia affinis.* S. W. GEISER (introduced by S. O. Mast), Johns Hopkins University.

Field collections of *Gambusia* and of other viviparous poeciliid teleosts almost invariably show a great preponderance of females. Experiments with litters of *Gambusia* raised in aquaria, with low mortality-rates, show the proportions of the sexes at birth to be approximately equal. The adult males have a higher death-rate than the females, thus producing a minority of males in adult populations. The gonads of the sexes at birth are indistinguishable from each other. They differentiate at ages of three weeks to over a year, depending upon environmental conditions. Temperature and food largely determine the rate of development. In my experimental litters, all gonads were differentiated in less than four weeks, so that ascertainment of sex was possible. Cytological study shows a fairly close correspondence between the degrees of differentiation and of the development of the anal fin (in the male) into an 'intromittent' or copulatory organ (gonopod). With favorable environment, the gonopod is developed in 95 per cent of the individuals within the first three months, but this development may be delayed till the fish is over a year old. The males in early stages (first 1½ to 2 months) grow in length and weight faster than the females. Total length in both sexes is trebled to quadrupled in the first three months. Females born in May, June bear their first litters of young when 8 to 10 weeks old. The chromosomes in soma and germ-cells are exceedingly small. Their spermatogonial number appears to be 36.

## G. PROTOZOÖLOGY

1. *Trophic and reproductive chromatin in the ciliate infusoria compared with similar conditions in other protozoa.* M. M. METCALF.

Euciliata have a trophic macronucleus and a genetic micronucleus. The Protociliata have in each nucleus a double series of chromosomes, one set genetic, the other trophic. In the Plasmodroma we often find two sets of chromosomes, one set introcentrosomal (genetic?), the other set extra-centrosomal (trophic?). Illustration and discussion of these conditions.

2. *Some effects of conjugation in the life-history of Spathidium spathula.* LORANDE LOSS WOODRUFF and HOPE SPENCER, Yale University.

A study has been made of about sixty lines of exconjugants, all derived from a pedigree culture of *Spathidium* and bred under identical cultural conditions. A comparison of the division rate and longevity of the parent lines and their exconjugant lines gave the following chief results. 1) A large majority of the exconjugant lines exhibited a higher division rate than their parent lines. 2) A large majority of the exconjugant lines continued to live after the death of the parent lines. 3) From the original parent cell to the  $F_6$  generation there have been nearly 1000 cell divisions, punctuated at the 30th, 134th, 425th, 573rd, 644th, and 904th divisions by fertilization. The survival value of conjugation is indicated by the fact that the parent,  $F_1$ ,  $F_2$ ,  $F_3$ ,  $F_4$ , and  $F_5$  generations have died in turn—the pedigree culture now existing in the  $F_6$  generation.

## H. PARASITOLOGY

1. *The parasitic Protozoa of Blatta orientalis and their value as the material for use in a class of parasitic Protozoa.* R. KUDO, University of Illinois.

One of the difficulties connected with the study of parasitic Protozoa lies in the collection of host animals. Artificial infection and cultivation of parasitic Protozoa have only been done in a comparatively few cases. At present one must obtain a great majority of parasitic Protozoa from their natural sources—the host animals. The occurrence of parasitic Protozoa is highly uncertain. Ordinarily a host species does not harbor many species of Protozoa and the per cent of infection is usually small under natural conditions. For their study, especially in a class-room, one has to collect many individuals of the host animals. The material desirable for a class work should at least fill the following conditions: a) the host animal should harbor Protozoa of variety and large dimensions; b) the per cent of infection should be high; c) the host animal should easily be collected and kept alive in the laboratory; d) the host animal should be of convenient size. After comparative studies of various animals as the host, I came to the conclusion that *Blatta orientalis* is so far best fitted for the purpose and recommend it as the material for class work. The insects examined by me at Urbana, Illinois, harbored the following species of Protozoa: *Endamoeba blattae*, *Lo-phomonas blattarum*, *L. striata*, *Coelosporidium periplanetae*, *Gregarina blattarum*, *Nyctotherus ovalis*. The papers of pioneer Protozoologists (Stein, Bütschli, Leidy, etc.) and of modern workers (Janicki, Mercier, etc.) upon these Protozoa will be reviewed elsewhere with my own observations upon them.

2. *A preliminary report on the activities of infective hookworm larvae in the soil.*

WILLIAM W. CORT and DONALD L. AUGUSTINE, Johns Hopkins University.

Little is known of the activities of infective hookworm larvae in their natural environment on account of the difficulty of studying them in the soil. An apparatus invented by Baermann, which has made it possible to isolate hookworm larvae from considerable quantities of soil, was used in investigations, which were carried on during the past summer in Trinidad, British West Indies. It was found that a large percentage of infective hookworm larvae isolated from soil samples taken in the field and from laboratory experiments had lost their protective sheaths, indicating that it is a very common thing for them to complete their second moult in the soil and to continue to live unsheathed. Both field studies and laboratory experiments showed that hookworm larvae do not migrate to any extent in the soil, so that soil infestation is practically limited to the places of development. It was also learned that the life of the infective hookworm larvae in the soil is much shorter than is usually supposed. In less than six weeks after the elimination of soil pollution from a cane field, the hookworm larvae present in the soil had almost died out. A series of laboratory experiments showed the same shortness of life even under the most favorable conditions. In these experiments even in three weeks there would be a considerable reduction in numbers and almost none of the larvae survived after six weeks.

3. *Notes on North American blood flukes.* G. A. MACCALLUM, Baltimore.

These interesting trematodes are comparatively new to science. Probably the first found in America were on January 4, 1912, when I found three small worms in the washings of the intestines of a *Chelopus inculptus* and which I named *Spirorchis*, but carelessly omitted the specific name which I intended to be *eustroptos*, both names meaning twisted or spiral, referring to the testis. Again on January 4, 1917, several more of the same were found, and shortly after a species was found in the intestines of *Chrysemys picta*, and these were named *S. pictae*, and what seemed odd some of *S. eustreptos* were also found in the intestines of *Chrysemys picta*, apparently the same worms as those found in *Chelopus inculptus*. Again on August 4, 1921, a specimen was taken from the lung of *Emys blandingii* quite different but of the same genus shown in *Zoopathologica*, vol. 1 no. 6. It is somewhat imperfect, but recognizable. Then on July 17, 1921, I found within the heart of a *Chelydra serpentina* (western form) five *Spirorchidae* which were attached to the wall of the ventricle, but all coiled together as if in coition. These worms were the largest of any *Spirorchidae* I had seen, being in length 8.50 to 9 mm.  $\times$  1 mm. wide, and which I have named *S. chelydrae*. The peculiarity about these worms is the much bent oesophagus, also the numerous glands at the junction of the oesophagus and coeca and possibly posterior also on the outside of the oesophagus to the mouth. These glands probably secrete a sort of hirudin which acts as an anticoagulant for their food.

4. *A study of diphylobothrium ova.* MEYER WIGDOR.

Magath ('19), on the basis of the measurements of 100 ova of *D. latum* and on a peculiar minute thickening of the shell at the pole opposite to the operculum, states: "With these characteristics, which are purely morphologic, one may make a very definite diagnosis of the presence of this worm, the egg lying within the

range of size given above." Measurements of 25 eggs of *D. latum*, *D. fuscum* and *D. pseudocordatum*, a new undescribed species, were made by me and the egg measurements of *D. cordatum* compared. The peculiar shell thickening described by Magath was noticeable in the three species in which eggs were available, so that this morphological feature is apparently a generic rather than a specific one. The ova of *D. latum* in man as given by Magath have an average ratio of length to width of 138:100. My measurements of the ova of *D. latum* and *D. fuscum* in the dog show ratios of 137:100 and 135:100, respectively. As far as the morphological characteristics and the size of the egg of the two species are concerned, *D. latum* and *D. fuscum* in dogs cannot be readily distinguished. The ova of *D. cordatum* and *D. pseudocordatum* are more elongated and relatively narrower than the preceding two species and can usually be differentiated from those two species if an average of several measurements is taken. There is a marked variation in the size and shape of the eggs, however, so that the largest egg of *D. latum* and *D. fuscum* can hardly be differentiated from the smallest eggs of *D. pseudocordatum*. *Diphyllbothrium* ova afford a generic, but hardly a specific differentiation.

5. *A new genus of trematodes from the white-bass.* H. J. VAN CLEAVE, University of Illinois.

A heavy and general infestation by minute distomid trematodes was discovered in the intestine of the white bass (*Roccus chrysops*) from the Mississippi River during an investigation of fish parasites for the Bureau of Fisheries. These trematodes belong to a new genus to which the name *Allacanthochasmus* has been ascribed and for which *A. varius*, the only certain species, is designated as type. *Distomum tenue* of Linton seems to have many points of structure in common with this new genus. Individual variation is very marked in this new species. Relative position of organs such as the testes are greatly modified by the degree of contraction or extension of the body. The oral sucker is surrounded by a prominent crown of spines arranged in a complete single circle. *Allacanthochasmus* seems to have definite relationships with the genus *Acanthochasmus* of Looss and may have considerable phylogenetic significance. Members of this genus display marked host specificity for extensive investigations of the parasites of other fishes, from the same localities where infestation of *Roccus chrysops* were general, failed to disclose a single instance of infestation in any species other than the type host.

6. *Apparent racial immunity to certain nematode infections.* BENJAMIN SCHWARTZ and MARCOS A. TUBANGUI, University of the Philippines.

In the course of a survey on the frequency of helminthic infections in man in the Philippine Islands that is still in progress, it has been found that while the percentage of infestation with certain common intestinal nematodes, namely, *Trichuris*, *Ancylostoma*, and *Necator*, is very high, the degree of infestation is extremely light. Out of 250 examinations, about 60 per cent were found to be positive for whipworm and hookworm. With very few exceptions, infestations with these nematodes, as judged by the scarcity of ova in concentrated sediments of fecal samples, were very light. The frequency of infestation with *Ascaris* has been found to be lower than that of whipworm and hookworm, but numerous

eggs have been found in positive fecal samples. These observations are in harmony with autopsy findings which show almost invariably light infestations with whipworm and hookworm and from moderate to heavy infestations with ascarids. Inasmuch as living conditions in the Philippines favor heavy helminthic infections, it may be concluded that Filipinos are comparatively immune to infestations with *Trichuris*, *Ancylostoma*, and *Necator* and that they are quite susceptible to infestations with *Ascaris*. Possibly an initial light infection with whipworm and hookworm establishes an immunity to further parasitization. The possible presence of such immunity in Filipinos is being investigated by serological methods.

7. *Intestinal protozoan infections as an index of personal hygiene and institutional sanitation.* WILLIAM C. BOECK, University of Wyoming.

Protozoan surveys conducted in England and a similar survey in this country completed by the U. S. Public Health Service on June 30, 1921, have shown that the incidence of protozoan infections was higher among persons confined in institutions than among those leading a free existence. Confinement was in some way responsible. As a part of the latter survey, 190 patients were examined from an asylum, 108 prisoners from a northern penitentiary, 77 training school boys, and 38 industrial school boys and girls. These persons had been admitted to their respective institutions since 1915. Another series comprises 545 patients who had been confined in the asylum from 1 to 42 years. The results of these examinations were: 1) The incidence of infection was found to increase with additional years of confinement. 2) a large number of pure (single) and a smaller number of mixed (double, triple, etc.) infections occurred among persons but recently admitted to an institution, while the opposite was true among persons who had been confined for longer periods. The number of pure infections became mixed later on by the acquisition of new infections. 3) The spread of protozoan infections seemed the result of low standards of personal hygiene and institutional sanitation. Less infections were found among the prisoners than among the other institutional cases, which was attributed to the excellent standards of personal cleanliness and institutional sanitation in the prison which did not obtain to same degree in the other institutions.

8. *Ophiotaenia testudo* nov. spec. from *Amyda (trionyx) spinifera*. THOMAS BYRD MAGATH, Mayo Clinic.

The description of a cestode found in the intestine of the soft-shell turtle (*A. spinifera*) from Lake Pepin, Lake City, Minnesota, is presented. This record is believed to be unique in the respect that no descriptions have been made in American literature of cestodes from turtles although many animals have been examined from the Mississippi River. Twenty turtles were examined, all or thirteen, whose carapaces were over 8 inches long harbored the parasite, those under 8 inches were not infected. The chief food of this turtle at Lake City is crayfish, which being the distinctive food of the soft-shell turtle suggested the crayfish as the intermediate host. Many were examined without success, as were attempts to experimentally infect them. The worm belongs to the genus *Ophiotaenia*, but is unlike any of the described species. Length 50 cm., maximum breadth 1.6 mm. Scolex, 0.63 mm. wide and 0.42 mm. long. A median fifth



sucker is absent. The four suckers are 0.12 mm.  $\times$  0.10 mm. Genital pore irregularly alternating at the end of the anterior sixth. Cirrus pouch, 0.56 mm.  $\times$  0.27 mm.; ductus ejaculatorius is coiled and the testes (about 200) are in two lateral fields. The vaginal opening is anterior to that of the cirrus. Ovaries, alated; vitellaria extend nearly to the anterior margin of the proglottid. The arrangement of the female genital organs is essentially like that of the other members of the genus. The uterus is median and has about fifteen pouches. The gross diameter of the eggs is 0.030 mm. This paper will be published as a contribution from the United States Bureau of Fisheries Biological Station at Fairport, Iowa, and the Section on Clinical Pathology, Mayo Clinic, Rochester, Minnesota.

9. *Otacariasis in the fox.* WM. A. RILEY, University of Minnesota.

Ear canker is a widely distributed and serious difficulty among domesticated foxes. Unlike that of rabbits, it is not due to Psoroptes, but to a species of Otodectes. Experimental and control work will be reported.

10. *A new venture in the field of practical parasitology.* (Motion pictures.)

B. H. RANSOM, U. S. Bureau of Animal Industry.

Recent discoveries concerning the life-history of *Ascaris lumbricoides*, a common and highly injurious roundworm parasitic in human beings and in swine, have suggested the possibility of minimizing the losses caused by this parasite among young pigs. At present in many localities these losses are very serious, to the great detriment of the live-stock industry. During the last two years some attempts at a practical control of roundworm infection have been made by the Bureau of Animal Industry of the U. S. Department of Agriculture on a considerable number of farms in the Middle West with highly successful results.

11. *The host-parasite method of investigation and some problems to which it gives approach.* M. M. METCALF.

Problems as to 1) Taxonomic relations between hosts; illustrated from Vernon Kellogg's work upon bird-lice. 2) Taxonomic relations between parasites; illustrated from Aphides. 3) Place, and time of origin and migration routes of hosts; illustrated from the genera *Hyla* and *Bufo* and their *Opalinidae*. 4) Place and time of origin of the parasites and their migration routes; illustrated from *Zelleriella* and *Protoopalina*. 5) Paleoclimatic conditions; illustrated from evidence from the present geographical distribution of *Anura* and *Opalinidae*, a) as to drought in northern Mexico, b) as to temperature and moisture in Antarctica. 6) Conditions of forestation and moisture in the past; illustrated from Central America and South America and a study of their *Hylids* with their *Opalinids*. 7) Ancient land connections; illustrated from the Americas and evidence from fishes Amphibia and their parasites; also from Johnson's work on Australian Cestoda and Trematoda. Comprehensive, concomitant studies of host and parasite among both animals and plants is of essential importance in reconstructing paleogeography in all its phases, and is of great value from other points of view.

12. *The rôle of domestic chickens and pigs in the spread of hookworm disease.* JAMES E. ACKERT, Kansas State Agricultural College.

Feeding experiments with domestic chickens and pigs, conducted last summer in Trinidad, British West Indies, demonstrated that these animals act as vectors in the spread of hookworm disease. By administering hookworm eggs in human excrement to chickens, it was shown that not only do such eggs remain viable in passing through the fowls, but they produce infective hookworm larvae in a medium of chicken feces and animal charcoal or soil. However, a series of quantitative tests indicated that a majority of the hookworm eggs swallowed by chickens fail to produce infective larvae. This failure was attributed to breaking of eggs in the gizzard, injury from urine in the feces, and to malnutrition of the larvae in the excrement. On the other hand, the experiments show that chickens may bring hookworm eggs from remote places up to the drinking dishes, and thus establish infective spots frequently traversed by the barefooted civilians. Newly hatched hookworm larvae pass through the digestive tract of chickens apparently uninjured, but infective larvae administered to chickens do not pass at once from the fowls. On the whole, the domestic chicken appears to be more beneficial than harmful in the control of hookworm disease in Trinidad. Unfortunately, not so much can be said for the pig whose masticatory, digestive and excretory systems so closely resemble those of man. Results of experimental feedings indicated that the majority of hookworm eggs swallowed by pigs produced infective larvae in a culture medium of pig feces and soil.

13. *A study of the resistance of different hosts to Trypanosome infections.* W. H. and L. G. TALIAFERRO, Johns Hopkins University.

The resistance which an animal offers toward a trypanosome infection may be directed either toward a retardation of the rate of reproduction of the parasites or toward their actual destruction after they are formed. We have been able to distinguish between these two conditions, first, by counting the trypanosomes per unit volume of blood and, second, by ascertaining their comparative rate of reproduction as is shown by their coefficient of variation for size. The use of the coefficient of variation as a measure of the rate of reproduction is making use of the obvious fact that a rapidly reproducing population of protozoa is more variable than one in which there is little or no reproduction. Its great value lies in the fact that its determination is independent of the actual number of organisms present. Studying the problem of resistance along these lines, we have reached the following conclusions: 1) in *T. lewisi* in the rat, a resistance develops which both retards the rate of reproduction of the parasites and destroys them after they are formed; 2) in *T. brucei*, etc., in guinea-pigs and dogs, the resulting resistance leads to the destruction of the parasites after formation with no appreciable effect on the rate of reproduction, and, 3) in *T. brucei*, etc., in rats, no resistance on the part of the host develops either toward their rate of reproduction or their destruction after formation.

14. *The anomalies in the group of blood-infesting trematodes.* HORACE W. STUNKARD, New York University.

The group of blood-inhabiting trematodes at the present time comprises three families, the Schistosomatidae, the Spirorchidae, and the Aporocotylidae. The

first of these families is the best known and contains the bisexual forms parasitic in the blood vessels of birds, cattle and man. The Spiroorchidae are hermaphroditic forms parasitic in the vessels of turtles, and the Aporocotylidae are also hermaphroditic occurring in the blood vessels of fishes. Probably no other trematode group presents such unusual variety and diversity, and no adequate explanation has as yet been presented for this condition. Common characteristics are found throughout the group, but within the families and even genera great morphological disparities are present. The fish parasites are without organs of adhesion, one genus of turtle parasites has only an oral sucker while the other has both acetabulum and oral sucker, and in the family Schistosomatidae there are genera without suckers and others with both ventral and oral adhesive organs. In the Aporocotylidae the genital pore is dorsal, in the Spiroorchidae it is ventral and somewhat lateral, and in the Schistosomatidae it is ventral, either in the median line or to the right or left of it. Within each family there are representatives with a long coiled uterus containing many eggs, and others with a short uterine portion of the female genital duct containing a single egg. These major differences are accompanied by minor differences and constitute a large and unusual list of anomalies in the group of blood infesting trematodes.

15. *Some additional results obtained in the study of infectious anemia of horses.*

JOHN W. SCOTT, University of Wyoming.

Principal results since previous reports include: 1) *Tabanus septentrionalis* can transmit the disease. 2) Saline tabanid extract produces anaphylactic reaction only. 3) Some horses have a high degree of natural immunity, show no apparent symptoms, and still are carriers of highly virulent blood. 4) The course and character of the fever usually serves to distinguish horses infested with parasitic worms from chronic cases of swamp fever. 5) Blood of one carrier has been virulent over five years. 6) Experiments have proved that the virus is present in the nasal secretion, thus suggesting new possibilities in transmission. 7) In the course of the disease, the erythrocytes gradually diminish but frequently regain the normal number, the hemoglobin content remains closely proportional to the number of erythrocytes present, and the leucocytes increase at a fever period. 8) With cooperation of Dr. S. H. Burnett, it was found that the total increase of leucocytes was due, chiefly at least, to increase in polymorphonuclears and that the eosinophiles disappear or greatly decrease during a fever reaction. In cooperation with Dr. F. S. Jones, of the Rockefeller Institute, contrary to findings of the Japanese, we were unable to find spirochaetes, 1) in stained blood smears or, 2), by using dark-field illumination, in fresh citrated blood either before or after centrifuging, in fresh smears of spleen, kidney, liver, and lymph gland, or in Noguchi cultures from such organs and from the blood. 3) A very minute rod-like organism was found in some cultures, nature unknown, since inoculations so far have given negative results.

16. *Sporulation and development of the cysts in a new species of Myxosporidia, Lentospora ovalis.* H. S. DAVIS, University of Florida.

Gills of the buffalo-fishes, *Ictiobus bubalus* and *I. cyprinella* from the Mississippi River at Fairport, Iowa, are often badly infected by a new species of Myxosporidia, *Lentospora ovalis*. The parasites are carried to the gills in the

circulating blood as amoeboid plasmodia containing three or four nuclei. These nuclei become differentiated into two distinct types, vegetative and generative, which have very different functions. Both types multiply rapidly, the generative much faster than the vegetative. The cysts normally develop within the blood vessels, the walls of which become greatly distended. Each pansporoblast develops from a single cell, the first evidence of the formation of the definitive pansporoblast being a heteropolar division which divides the cell into two very unequal parts. The daughter cells remain closely associated after division and from them are derived all the cells of the pansporoblast. The smaller cell divides but once to form the two envelope cells which become flattened and form a thin covering for the remaining cells of the pansporoblast. Each pansporoblast forms two spores of six cells each. In late stages of the pansporoblast small deeply staining chromatoid bodies are often present. Their origin was not determined but it is not believed they are reduction nuclei since their number and size is very inconstant. No evidence was obtained of any sexual process during the development of the spores. The spores continue to develop until the cysts reach a diameter of about 500 to 700 $\mu$ .

17. *On the migration of the sheep lung worm, Dictyocaulus filaria.* JOHN E. GUBERLET, Oklahoma Agricultural Experiment Station.

The writer ('19), in the case of *Dictyocaulus filaria*, has shown that infestation takes place through the mouth and that the larvae migrate to the lungs. It was thought for a time that the migration of lung-worm larvae took place as in *Ascaris*, as has recently been shown by Stewart ('17, '19), Ransom and Cram ('21), and Yoshida ('19). Recent experiments with *dictyocaulus* in which gross infestation was produced by giving numerous larvae almost daily over a period of twenty days have thrown some light on the matter. At the end of twenty days the lamb was killed and autopsied. Numerous larval worms were present in the blood. Mesenteric lymph glands, liver, spleen, and kidneys, as well as lungs, contained larval worms. The mesenteric lymph glands were much enlarged. Previously, it was observed that lung symptoms first appeared about twenty days after inoculation. In this case no symptoms appeared until the eighteenth day. Blood was occasionally drawn from the jugular vein, the last being taken on the fourteenth day, with negative results. Apparently, the larval worms do not enter the blood stream at once, but remain in other organs, very probably in the mesenteric lymph glands for some time before entering the blood which carries them to the lungs. The presence of larvae in the liver, spleen, and kidneys is undoubtedly a late development and due to the circulation. Subcutaneous inoculation with larval worms gave negative results.

18. *Studies on Spirochaeta duttoni in the tissues of its invertebrate host, Ornithodoros moubata.* E. MELVILLE DU PORTE, Macdonald College, McGill University.

The spirochaete was found to be still present in three races of laboratory-reared ticks brought to this country originally in 1913. Sections of the ticks show the presence of the spirochaete in most of the organs and tissues including the cephalic glands, brain, mesenteron, uterus, ova, and the connective, adipose, and muscular tissues. The eggs may become infected within the ovary or in their passage through the uterus. Eggs that escape infection within the body of

the tick may be infected after being laid since the secretion of the cephalic gland, with which they are covered, may contain large numbers of spirochaetes.

19. *A little-worked source for parasite material.* M. M. METCALF.

Museum specimens of animals preserved in alcohol give workable material of many internal parasites, especially nematodes and ciliate infusoria. Formalin material is unsatisfactory. The generous attitude of the United States National Museum. The importance of using such material in many museums. It is important in collecting, that both the larger organisms and their parasites should be in mind in deciding methods of killing and preservation.

20. *A coelomic coccidian of Tribolium.* WM. A. RILEY and LAURENCE KROGH, University of Minnesota.

While Coccidia are widely distributed and numerous in species, coelomic forms have been rarely noted. Three species have been reported for insects and two for Oligochaetes. An apparently new species has been found abundantly in flour beetles belonging to the genus *Tribolium*. Life-history studies and infection experiments are to be reported.

21. *On Leptotheca ohlmacheri, a myxosporidian parasitic in frogs.* R. KUDO, University of Illinois.

About 40 per cent of *Rana clamitans* and *R. pipiens* studies were found to be infected by a myxosporidian, *Leptotheca ohlmacheri*. The spores had been known since Ohlmacher ('93) found them in the kidney of *Bufo lentiginosus*. The seat of infection is the lumen of the uriniferous tubule and the space between Bowman's capsule and the glomerulus of malpighian body of the kidney. The infection of a new host animal seems to take place per os. The spore germinates in the stomach under the influence of gastric fluid. The amoebae penetrate through the intestinal wall and pass into the coelom, hence into the uriniferous tubule or malpighian body by way of nephrostome or blood vessels. The spore contains two independent uninucleate sporoplasms which fuse into one mass at the time of germination. The nuclei seem to unite later. The young stage found in the tubules of the kidney is small uninucleate form. Schizogony in this stage seems to be of probable occurrence. The uninucleate form grows. The nucleus divides into two, one vegetative and the other generative nucleus. The former by undergoing further divisions produces trinucleate gemma which becomes separated from the mother body. The latter divides into two generative nuclei, each of which develops into a spore. The trophozoites are usually disporous. The vegetative nucleus persists throughout the entire trophic life of the trophozoite. The parasite does not seem to exercise fatal effect upon the host animal.

22. *Notes on two new species of holostomidae.* JOHN E. GUBERLET, Oklahoma Agricultural Experiment Station, Stillwater.

Two species of Holostomidae were found in the intestine of a loon (*Gavia immer*) killed near Stillwater, Oklahoma. These proved to be undescribed species belonging to two genera, *Hemistomum* Diesing ('50) and *Strigea* Abildgaard (1790). For the former the name *Hemistomum gavium* is proposed. This

is a small form 1 to 1.5 mm. in length. The flattened anterior region, 0.30 to 0.40 mm. in breadth and 0.094 mm. in thickness, comprises approximately two-thirds of the body length. The adhesive disk is posterior to the acetabulum, but does not cover it. The posterior cylindrical region is 0.24 to 0.28 mm. in diameter. For the species in the latter genus the name *Strigea aquavis* is proposed. This species is 2.5 to 3.5 mm. in length. The cup-shaped anterior region measures 0.5 to 0.75 mm. in length and 0.6 to 0.9 mm. in diameter. This region contains the oral sucker, acetabulum, and adhesive disk, or hold-fast organ. The oral sucker and acetabulum are approximately of equal size. The hold-fast organ is composed of leaf-like structures which protrude anteriorly. The cylindrical posterior region, 0.45 to 0.55 mm. in diameter, contains the reproductive organs. Heretofore, only one North American species of *Hemistomum* has been recorded, that being *Hemistomum craterum* Barker and Noll ('15), from *Fiber zibethicus*. The only species of the genus *Strigea* previously reported from North America is *Strigea cornu* Rud., from *Ardea herodias* by Stiles and Hasall ('94).

#### I. COMPARATIVE AND GENERAL PHYSIOLOGY.

1. *Mutual reactions of cell-bodies and pseudopodial fragments of Diffugia.* WM. A. KEPNER and B. D. REYNOLDS, University of Virginia.

1. In *Diffugia* we have non-nucleated ectoplasmic fragments fusing with the parent cell, resulting in restitution. 2. The parent cell and ectoplasmic fragment are guided to each other by some chemical means other than carbon dioxide, or urea. 3. Fusion never occurs at the ends of the parental pseudopod, but is always effected along an extended midregion; therefore, there must be a difference between the ends and the middle of such pseudopods. 4. The response between a severed fragment and the parental cell is mutual, the direction of approach being shifted to correspond to the changing positions of the protoplasts involved. Movement on the part of an enucleated ectoplasmic fragment seems to be more than aimless. 5. All mutual reactions between protoplasmic fragments and cells were found to be individual rather than specific. Our observations indicated that if an individual protoplasm be separated there would be a tendency to restitution of the whole; but even though one cell be deprived of a part of its body, if protoplasm of another species, or from another individual of the same species, be placed in proximity to it, there will be no response. Thus it is emphasized that after all there is something peculiar in the individual's make up. This peculiarity may be due to the allotment of germ-plasm assigned to the individual at cell division, or to the changes wrought by the flux of the life of the individual since separation from its parent, or both.

2. *Reactions to light of the earthworm, Lumbricus terrestris.* WALTER N. HESS, DePauw University.

Earthworms, which are negative to light of ordinary intensities, become positive, in keeping with their nocturnal habits, when the light is greatly diminished. If, however, the brain is removed either by a dorsal incision or by the removal of the first three anterior segments, they no longer react negatively to ordinary illumination, but become strongly positive, and if six or even more of the anterior segments are removed, they are still positive. Similar results were obtained with

*Allolobophora foetida* with as many as forty anterior segments removed. These results show that the brain of the earthworm is not necessary for reactions to light and photic orientation. They indicate that earthworms are more sensitive to light when the brain and the photoreceptors at the anterior end are functional, than they are when they are not functional. This accounts for the fact that, while normal worms are positive only in very weak light, specimens with the brain removed are positive in strong light.

3. *The rate of metabolism of Paramecium with controlled bacterial food supply.*

GEO. T. HARGITT and RUTH L. PHILLIPS, Syracuse University.

It is easy to sterilize *Paramecium* so that no bacteria remain adhering to its body, and by using ordinary bacteriological technic it is also feasible to control the bacterial content of a culture fluid. The two methods combined enable one to control the food of *Paramecium* experimentally, as rigidly as desired. The rate of metabolism of *Paramecium* has been tested under these conditions of controlled bacterial food. The bacteria used were taken from ordinary hay infusions and used either in pure cultures or as mixtures of 2 or 3 kinds. All experiments were checked by the metabolic rate of *Paramecium* fed on the usual unknown bacterial mixture of the hay infusion. Some of the experiments covered only a few days or weeks, others extended over a period of six months, and two were observed for nine months.

In general the highest rate of metabolism seems to occur in such complex mixtures of bacteria as are found in hay infusions. The single pure culture of bacteria which was successful gave a low rate of metabolism for *Paramecium*, but the rate was fairly uniform. Mixtures of bacteria gave excellent results with a rather high rate of metabolism, though usually not so high as with the more complex mixtures of the hay infusion. Tests were also conducted upon the effect of changing the bacterial food from time to time. It was concluded that if the food was satisfactory at first a continuance of this food gave higher and more constant rates of metabolism than a change of food.

4. *The feeding reactions of Amblystoma tigrinum (Green).* ALBERT KUNTZ and JOSÉ ZOZAYA, St. Louis University School of Medicine.

This paper comprises the results of experimental work carried out at the Macbride Lakeside Laboratory during the summer of 1920 which may be summarized as follows: The typical response of the larvae of *Amblystoma tigrinum* to the presence of food consists of an 'approaching' and a 'seizing' reaction. Young adults frequently exhibit also a 'nosing' reaction. The 'approaching' reaction is commonly a visual response. The 'nosing' and 'seizing' reactions, except when the latter follows the 'approaching' reaction more or less spontaneously, involve the olfactory sense. Moving objects are detected and pursued more promptly than objects which are not in motion. In the absence of sight food is detected by the sense of smell. Discrimination between edible and inedible objects also involves the olfactory sense.

5. *The rôle of the nervous system in the locomotion and regeneration of marine polyclads.* J. M. D. OLMSTED, University of Toronto.

The locomotion of polyclads is comparable with that of gastropods. Ataxic locomotion is a local phenomenon, but controlled by the nervous system since

it is abolished by chloretone. Certain species progress only by this method. Ditaxic retrograde locomotion is under the control of the cephalic ganglia, each ganglion governing the progression of muscular waves on its own side. The nerve cords serve as conductors for impulses leading to wave formation each on its own side. Cutting one nerve causes the waves to disappear on that side at the level of the cut. Ciliary action is not under the control of the nervous system and plays practically no part in locomotion. For the swimming movement it is necessary that the cephalic ganglia be intact. Injury to or loss of the brain prevents the animal from employing this method of locomotion. Polyclads are able to restore missing parts provided the cephalic ganglia are intact. If these are injured nervous tissue is not added to restore the brain to its original size, and if they are entirely removed regeneration can not take place anteriorly, though it may do so posteriorly. If a portion of the brain remains, a certain amount of anterior regeneration takes place and eyes are regenerated, but not enough material is added to restore the original form, and the new eyes never reach the size of the old ones.

6. *A quantitative study of tarsal sensitivity to solutions of saccharose in the Red Admiral butterfly, Pyrameis Atalanta (Linn).* DWIGHT E. MINNICH, University of Minnesota.

The author has previously shown that the tarsi of the red admiral butterfly, *Pyrameis atalanta* (Linn), are sensitive to contact chemical stimulation. One substance sharply distinguished through the tarsi is a 1M solution of saccharose. If the tarsi are brought in contact with this solution *Pyrameis* always responds by extending the proboscis, irrespective of its nutritional condition. The animal may also respond if the tarsi are brought in contact with distilled water, but only after a more or less prolonged period of total inanition. Moreover, the administration of water immediately inhibits the response. It is possible, therefore, to maintain *Pyrameis* in a state of 100 per cent responsiveness to 1M saccharose, but of 0 per cent responsiveness to water alone. Under these conditions the minimal concentration of saccharose necessary to effect a response, i.e., the threshold concentration, is readily determined. Seven butterflies were intensively studied under varying nutritional conditions. The results show that the threshold of response to saccharose varies directly with the nutritional condition. During periods of inanition with respect to saccharose the threshold gradually falls, and may reach such levels as M/3200, M/6400, or even M/12,800. But with the initiation of a period of saccharose diet, the threshold rises abruptly to a level usually of about M/10, where it remains approximately constant as long as the diet continues. Compared with other animals, the sensitivity to saccharose in *Pyrameis* is very highly developed. This is doubtless correlated with the fact that sugars form the chief food of this insect.

7. *A micro-Winkler method for quantitative determination of dissolved oxygen.* E. J. LUND, University of Minnesota.

It has been found possible and entirely practicable to determine dissolved oxygen in quantities of water of 10 cc. or even less by employing 0.1 cc. each of the solutions KI-NAOH, and  $MnCl_2$  ordinarily used in the Winkler method, instead of 1 cc. of each of these solutions. The thiosulphate solution of the



usual concentration is diluted ten times or even more if necessary. Addition of the reagents is best accomplished from micro-burettes, drawn to long capillary points. Titration in a small, deep dish is carried out with a burette reading to 1/20 cc. Loss of iodine is proportionately not greater than in the usual method. The limit of error is about 0.005 cc. or 0.007 of a milligram of oxygen gas. This procedure represents, therefore, by far the most delicate method ever devised for the quantitative determination of oxygen. Special procedures are used to remove protozoa, small pieces of tissue, etc., from the water before analysis. A full account of the methods will appear in the publications of the Puget Sound Marine Biological Station.

8. *A contribution to the colloid chemistry of protoplasm.* L. V. HEILBRUNN, University of Michigan.

Recent work on colloid chemistry has shown that the electric charge on the particles of colloidal solutions is of especial importance. Primarily it is this charge that keeps the colloidal particles dispersed through the liquid that contains them. Some colloidal solutions are positively charged, others negatively. It is essential to determine whether protoplasm is a positive or negative colloid. This can be done by an indirect method. Sea-urchin eggs were placed in isotonic solutions of various salts and then after varying intervals of time the protoplasmic viscosity was compared. Calcium chloride tends to liquefy the protoplasm, magnesium chloride also acts in this way, although less strongly; on the other hand, potassium, sodium, and ammonium chloride tend to precipitate protoplasm and their effect increases in the order named. The results indicate that protoplasm is positively charged. But the surface layer is negatively charged, for the very salts which coagulate the protoplasm cause the plasma membrane to swell, and those that liquefy the protoplasm tend to prevent this swelling. The results not only furnish evidence as to the charge on the colloidal particles of protoplasm, but they also provide an easy and rational explanation of the phenomena of antagonism.

9. *Transplantation of the pars nervosa of the pituitary.* W. W. SWINGLE, Yale University.

Subcutaneous homoplastic and heteroplastic transplantation of the pars nervosa of adult *R. clamata* and *R. catesbeiana* into larvae cause shrinkage and emaciation after twelve hours. Control grafts of the pars anterior and brain tissue show no such effect. The larvae vary in their reaction to the graft, but in none does the shrinkage persist following resorption of the implanted tissue. The experiments indicate that the shrinkage and emaciation of the tadpoles are due to the well-known diuretic action of pars nervosa secretion, and not to muscular contraction. In frog larvae the activity of the mesonephros is intimately associated with maintenance of normal body appearance. Tadpoles constantly absorb water through the skin, circulate it through the lymph and blood system finally eliminating it by means of the mesonephros. A continual stream of water passes through the organism; the tissues, body cavity and lymph sinuses are turgid, and it is owing to this fact that under normal conditions the tadpole body has a round oedematous appearance. Processes of absorption and elimination are in equilibrium, but any block in the kidney function or undue

stimulation of renal activity overthrows the equilibrium and the appearance of the larva changes. Blocking the kidney function leads to rapid and fatal oedema; transplanting the pars nervosa, the secretion of which apparently acts upon the larval mesonephros as it does on the mammalian metanephros by stimulating the secretory cells to greater activity, leads to shrinkage and emaciation owing to loss of turgor through increased elimination of water.

10. *Photic orientation in the robber-fly, Proctacanthus.* S. O. MAST, The Johns Hopkins University.

Specimens with middle and front legs on one side removed orient fairly accurately.

If the upper surface of one eye and the lower surface of the other are covered the insects, if properly illuminated, lean toward the former and turn toward the latter. If one eye is covered and the illumination is confined to a small portion of the other, the legs on both sides respond in such a way as to tend to cause the animal to turn toward the illuminated surface, no matter where it is located. If the illumination is confined to a certain portion of the anterior surface the insect goes fairly directly toward the light, although only one eye receives light. In light from two sources of unequal intensity the two eyes are also unequally illuminated when the insects are oriented.

The turning effect of the illumination of a given region of the retina on one side is neutralized by equal illumination of the same region of the retina on the opposite side or by lower illumination of regions relatively farther back.

These facts and others prove conclusively that the tonus hypothesis, or any other that demands balanced action in receptors and locomotor appendages on opposite sides, does not fully account for orientation in *Proctacanthus*. They show that it is dependent upon series of coordinated reflexes in the legs on both sides specifically related to the localization of the stimulus in either eye and inhibition of the effect of illumination in one eye by simultaneous illumination in the other.

11. *Light and growth in animals.* A. G. HUNTSMAN and F. FRASER, University of Toronto.

The growth of the mussel (*Mytilus edulis*) was found to be greatly impeded by exposure to full sunlight. This effect was shown to a greater degree in the antero-posterior and the dorsoventral than in the transverse axis or than in the weight of dry material. A comparison has been made of growth in full direct sunlight with that in direct sunlight diminished to about one-eighth. The rock barnacle (*Balanus balanoides*) shows nearly twice as much increase with the lower illumination. Colonies of *Clava leptostyla* show more than twice as great an increase in the number of zooids with the lower illumination.

12. *'Reversal of inhibition' in insects by atropine.* W. J. CROZIER, Rutgers College.

Representatives of a number of invertebrate phyla have been found to show under influence of strychnine a special excitation of extensor neuromuscular elements, such that there appear phenomena of opisthotonus and of reversal of inhibition. With insects strychnine is singularly ineffective in these respects,

and is of very slight toxicity. Sphingid and other large caterpillars, however, do show pronounced neuromuscular 'reversals' in connection with the antagonistic muscles controlling movements of the prolegs, but only when atropine has been injected; none of a series of neuropile substances, save atropine, acts in this manner. Annelids do suffer differential neuronc excitation under strychnine, as can be demonstrated very completely in leeches. With crayfish, strychnine is very slightly toxic, but does stimulate certain neuromuscular groups (leading to forward progression), and may produce opisthotonus. These facts point to chemical peculiarities of the insect nervous system, differentiating it from that of groups phylogenetically akin; and consequently have a direct bearing upon the conception of progressive chemical specialization of the nervous system.

13. *Electrical polarity in Obelia.* E. J. LUND, University of Minnesota.

A definite difference of electrical potential occurs in the stem of the colony of *Obelia*, such that the apical growing region is electronegative to the middle or more basal region. This electrical polarity is associated with the living tissue of the coenosarc and does not originate in any other structure of the stem for on potential difference occurs in: *a*) stems left to die and macerate in sea water; *b*) stems from which the living tissue has been removed mechanically, and, *c*) stems in which the living tissue has been killed by chloroform. The magnitude of the fall of potential varies in pieces of stems from different colonies. It also varies along the length of the stem of the same colony, being greatest in the apical actively growing region. The general conclusion from the experiments is that, since normally inherent differences of electrical potential occur in the stem of *Obelia* and are associated with apical growth, then it should be possible to inhibit or modify developmental processes by appropriate application of an E. M. F. of external origin. This was shown to be possible in the previous paper reported to this society a year ago.

14. *Circus movements in negatively heliotropic Limax.* W. J. CROZIER and W. H.

COLE, Rutgers College and Lake Forest College.

Analyses are already available of circus-movements executed by positively heliotropic organisms under suitable conditions, and the significance of these movements for the theory of heliotropism is generally recognized. We are able to show that in the negatively heliotropic slug *Limax*, with non-directive illumination, the animal circles continuously toward that side on which tip of the eye-tentacle (including the eye) has been removed. The diameter of the circular path varies inversely with the light intensity. It is found that the eyes, or structures in their immediate vicinity, are the effective receptors; upon regeneration of the eye following unilateral amputation, there is progressive return of typical orientation.

15. *The effect of chemicals on locomotion in Ameba. I. Reactions to localized stimulation.* J. GRAHAM EDWARDS (introduced by S. O. Mast), The Johns Hopkins University.

Localized stimulation was obtained by allowing various chemicals to diffuse from a capillary pipette against the surface of the ameba. The response depends

upon the composition of the medium in which the ameba is when stimulated. The following results were obtained from observations of specimens in N/500 KCl. In this solution they travel uniformly and monopodally. Acids (N/5 to N/500) cause a violent positive response. A protuberance is formed at the point stimulated which persists until incorporated in the folds at the posterior end; in lower concentrations, a small pseudopod is initiated which, however, does not persist. The response is in all cases more immediate and larger the nearer the anterior end the stimulus is applied, being negligible at the posterior end. The effect apparently depends on a local swelling of the ectoplasm with subsequent gelation. Hydroxides (N/5 to N/500) cause a broad sheet of protoplasm to flow toward the pipette, after which streaming continues in another direction. In lower concentrations, a small pseudopod is formed but does not persist. The effect here, as in acids, seems dependent on swelling of the ectoplasm, but without subsequent gelation. Salts induce various responses depending on their concentration and composition. Local changes in permeability seem to condition these responses. Alkaloids and non-electrolytes induce negative responses. If the leucobase of methylene blue is applied anteriorly, the endoplasmic flow stops and the ectoplasm wrinkles at the point of application. If applied laterally, an indentation is formed at the point of application and the ameba becomes crescent-shaped.

16. *A quantitative study of the reactions to light in Amoeba.* HARRY T. FOLGER (introduced by S. O. Mast), The Johns Hopkins University.

Amoeba reacts to sudden increase in illumination by complete cessation of movement. The time from the beginning of stimulation until this cessation occurs varies with the intensity of the light. It becomes shorter as the intensity increases. The decrease in reaction-time, however, is not proportional to the increase in intensity. The amount of light-energy (meter-candle-seconds) received during the reaction-time is greater in higher than in lower intensities. To secure a response, exposure for only a definite portion of the reaction time is necessary, the reaction taking place at precisely the same time as if the illumination had been continued. Thus the reaction-time may be divided into two parts: a) a stimulation-period during which the animal must be exposed to light to get a response; b) a latent period during which exposure is not necessary. The latent period varies greatly in different animals, under the same environmental conditions. Usually it is practically constant in any individual for a moderate length of time. However, there are occasional fluctuations which sometimes take place gradually but which may occur suddenly. Such changes in the latent period amount in some cases to more than 100 per cent and are apparently independent of any change in the environment. The stimulation-period varies inversely with the intensity of illumination. The light energy received during this period is constant. Dark adaptation is necessary for a response. This process usually requires less than one minute.

17. *The influence of temperature on the rate of locomotion in Amoeba.* ALPHONSE M. SCHWITALLA (introduced by S. O. Mast), St. Louis University.

The relation between temperature and the rate of locomotion in Amoeba was studied quantitatively by means of a modified Pfeiffer stage and thermocouples.

At constant temperatures there are alternate periods of acceleration and retardation in the rate of locomotion. These periods are readily interpretable as phases in a locomotor rate-rhythm. They are coincident with eruptive and quiescent periods of activity as manifested by the rate of flow in the endoplasm. The relation between the two phases is constant under certain conditions but not under others. The average rate of locomotion increases with a rise in temperature until a maximum is reached at about 25°, after which it decreases. The value of the van't Hoff temperature coefficient ( $Q_{10}$ ) for a given individual under different conditions is extremely variable, but not more so than it is for certain other physiological processes. The evidence points to the conclusions that there is no direct quantitative relationship between temperature and the rate of locomotion, and that temperature affects the rate of locomotion only through its effect on the rhythmical processes which condition locomotion.

#### J. UNCLASSIFIED

1. *The National Research Council Information Service.* ROBERT M. YERKES.

Under this title is offered a brief account of the informational clearing-house for science and technology which is being developed by the National Research Council. The 'Service' is maintained with the cooperation of investigators and for their use. Ordinarily they may consult it without charge. The organization will serve also to disseminate intelligible and reliable information concerning scientific research and the applications of its results. All who are interested in research or its products are invited to make use of the Research Information Service.

#### ADDITIONAL ABSTRACTS

The following abstracts were received too late to be included in the Abstracts printed before the meeting. The Society voted to receive these and ordered them printed as a part of the proceedings of the meeting. Six of the papers were presented in full at the meetings.

A'. 25. *A lethal gene which changes the order of the loci in the chromosome map.*

H. J. MÜLLER, University of Texas. Abstract too long to be printed.

C. 9. *The spermatogenesis of man.* THEOPHILUS S. PAINTER, University of Texas.

A study has been made on the spermatogenesis of man using testicular material (from two negroes and a white man) which was perfectly fresh when preserved. The main results are as follows:—there are 48 chromosomes in dividing spermatogonia in both the white and the negro testes. Two of these 48 chromosomes have no synaptic mates of the same size or shape. These are the X and Y sex chromosomes. Primary spermatocytes show 24 chromosomes. The X-Y sex chromosome consists of an element, the two halves of which are very unequal in size. When division occurs, the X chromosome goes to one pole, and the Y to the other.

C. 10. *The spermatogenesis of the opossum.* THEOPHILUS S. PAINTER, University of Texas.

The complete spermatogenesis of the opossum has been worked out, the points of main interest being, (a) the diploid or somatic chromosome number is 22. (Previous investigators have reported 17 and 24.) Two of these have no synaptic

mates of the same size and shape. They are the X and Y sex chromosomes. The X and Y components segregate to opposite poles during the first maturation division, hence half the sperm carry an X and half a Y sex-chromosome. A study of embryos confirmed the above conclusions.

- E. 10. *Notes on the Crocodilia of British Guiana.* ALBERT M. REESE, University of West Virginia.

Of the four species of Caiman described from British Guiana probably only one, *C. niger*, is dangerous to man, though native hunters consider all of the so-called "alligators" dangerous.

Since their hides are not suitable for leather the caimans are not subject to the chief source of danger that has thinned the ranks of the Florida alligator, but they are fairly scarce in some parts of the colony, while very abundant in other parts.

They are extensively killed, on some sugar estates, though it is doubtful if they are not more beneficial as destroyers of rodents than harmful in other ways.

Their nests resemble in form and location those of the Florida alligator, but are often built of finer and entirely "dead" vegetable material.

The eggs are usually smaller (those of *C. niger* were not found) and have usually, though not always, rougher, harsher shells than the eggs of the Florida alligator. The average number of eggs per nest is about 27. About 6 per cent of the eggs examined were infertile. The eggs are mostly laid in the late spring, and hatch in two or three months, the period of incubation probably varying with conditions of temperature, moisture, etc.

- E. 11. *A noteworthy adaptive modification of the webs of a siamese argiopid spider.* HENRY E. CRAMPTON, Barnard College.

Along the railroad through the jungles of southern Siam, the telegraph wires provide spaces of varying width in which Argiopid spiders construct their webs. The animals probably belong to the genus *Nephila*. Full-sized webs are built where the intervals are suitable. When the interspaces are less than the diameter of a normal web, the spiders modify the structures by omitting an upper sector of greater or less extent, according to an invariable mode of adjustment.

- E. 12. *On the distribution and ecology of the species of Partula inhabiting the Mariana Islands of the Western Pacific Ocean.* (Illustrated with lantern-slides and specimens.) HENRY C. CRAMPTON, Barnard College.

In the course of a recent journey, the species of *Partula* inhabiting Guam and Saipan were investigated, as in earlier studies in Polynesia proper. The general principles of distribution were confirmed, as regards inter-island and, intra-insular differentiation. The environmental influences, as such, are not determinative with reference to the distinctive characters of distinguishable species and subordinate varieties.

- H. 23. *Phases in the life-history of a holostome, Cyathocotyle Orientalis, nov. spec., with notes on the excretory system of the larva.* ERNEST CARROLL FAUST, Parasitology Laboratory, Department of Pathology, Peking Union Medical College, Peking, China.

1. A larval holostome, *Tetracotyle orientalis*, nov. spec. is described from Peking, China.

2. The excretory system of the tetracotyle consists of two parts, a close network of canals with granular inclusions (the reserve bladder), and the system of tubules, capillaries and flame-cells.

3. The analysis of the excretory system of the tetracotyle shows a development along an exact plan, with five main tubules on each side of the body, each tubule draining a system of 32 dorsal and 32 ventral capillaries and flame-cells. Each of these sub-systems is based on a 5-fold dichotomy. The formula  $\alpha \div \beta \div \gamma + il + \epsilon$  exactly fits such a system. In the adult worm there will probably be an exact multiple of this least common denominator.

4. Of six young ducks carefully examined for infection beforehand, one was used for experimental feeding with the tetracotyloform larvae. Twelve days after the feeding the experimental animal died and at autopsy 75 holostomes were recovered from the ceca. The controls were all negative. The worms are believed to be responsible for a diarrhoeic and possibly dysenteric involvement of the ceca.

5. Some of the worms recovered from the experimental host were adults while others were immature. Study of the adults showed the worms to belong to the genus *Cyathocotyle*. The species is new and is designated as *Cyathocotyle orientalis*.

6. Comparison of tetracotyle, immature and adult worms indicates the progressive changes involved in its growth and gives an insight into certain relationships of the genus and of the group.

I. 19. *The instincts and adaptations of several species of Tortugas fishes.* W. H. LONGLEY, Goucher College, Baltimore.

The local distribution of many species of fishes is determined by the character of the bottom. This is particularly true of what may be called the "sand-patch association," members of which require a substratum of a peculiar character in which they may hide temporarily or construct convenient shelters. *Opisthog-nathus* and *Gnathypops* inhabit vertical burrows, sometimes a foot and a half deep and lined with bits of coral and shell as well as lined with brick. *Toglossus* has unlined burrows more irregular in shape. *Xyriethys* simply buries itself in the sand when alarmed although there is reason to believe that after its excursions in the open it may return again and again to one place.

*Thalassoma nitidus* is commonly seen pecking at the surface of larger fishes presumably cleaning ectoparasites from them. These larger fishes return again and again to submit to the process of grooming and will drive away others which interrupt the process.

This *Thalassoma*, whose attentions are not merely tolerated but actually welcomed, is resembled in a striking way by a small blenny, which although rare occurs with it and is notably different from the other blennies in respect to certain reactions which accentuate its superficial resemblance to *Thalassoma*. The relation between the two species appears comparable to a certain extent with the so-called 'Batesian mimicry.'

Slides were used to show peculiarities of form and behaviour of members of the "sand-patch association" and *Thalassoma* in action. Others illustrated the obliterative effect of the changeable coloration of common species of reef-fishes.

- I. 18. *The reflex produced by chemical stimulation of the deeper respiratory passage.* E. HORNE CRAIGIE, Department of Biology, University of Toronto.

The respiratory and circulatory reflexes aroused by the projection of an irritant vapour into the trachea, bronchi, and lungs have been stated by certain observers to be obliterated by double vagotomy, and by others to be unaffected by this operation.

The respiratory response obtained in dogs is found to consist of increased expiratory effort and an inhibition of inspiration.

The vaso-motor response consists of a rather gradual fall in blood-pressure, followed at once by a somewhat slower return to normal. In some cases the fall was preceded by a slight, brief rise in pressure.

Both the respiratory and the circulatory reflexes are entirely unaffected by section of both vagi, which therefore cannot contain the afferent fibers concerned.

- I. 20. *Hen feathering induced in male fowls by feeding thyroid.* BENJAMIN HORN-ING and HARRY BEAL TORREY, University of Oregon Medical School.

When fed thyroid daily, in doses increasing with their weight, from the age of three weeks, our male Rhode Island Red chicks developed plumage of the female type, although males of this breed ordinarily do not pass through a juvenile stage characterized by plumage of the female type. The plumage of capons, usually ultra male, is not affected by thyroid feeding. Castrated females which typically develop male plumage are similarly unaffected. The primary effect of the thyroid feeding seems to be to increase the activity of the 'luteal' interstitial tissue of the testis. The end result is a hen feathered male resembling in all essential characteristics of the plumage the hen feathered males of the sea-bright bantam and campine breeds.



# AMERICAN SOCIETY OF ZOÖLOGISTS

## CONSTITUTION, OFFICERS AND LIST OF MEMBERS OF THE SOCIETY

### CONSTITUTION

#### ARTICLE I

##### NAME AND OBJECT

*Section 1.* The Society shall be called the "American Society of Zoölogists."

*Sec. 2.* The object of the Society shall be the association of workers in the field of Zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical investigation in this country.

#### ARTICLE II

##### MEMBERSHIP

*Section 1.* Members of the Society shall be elected from persons who are active workers in the field of Zoölogy and who have contributed to the advancement of that science.

*Sec. 2.* Election to membership in the Society shall be upon recommendation of the Executive Committee.

*Sec. 3.* Each member shall pay to the Treasurer an annual assessment as determined by the Society. This assessment shall be considered due at the annual meeting and the name of any member two years in arrears for annual assessments shall be erased from the list of members of the Society, and no such person shall be restored to membership unless his arrearages shall have been paid or he shall have been re-elected.

*Sec. 4.* Foreign Zoölogists, not members of this Society, may be elected Honorary Fellows upon unanimous recommendation of the Executive Committee by a majority vote of the members present at any meeting of the Society. Honorary Fellows shall not be required to pay dues.

#### ARTICLE III

##### OFFICERS

*Section 1.* The officers of the Society shall be a President, a Vice-President, a Secretary and a Treasurer and the members at large of the Executive Committee.

*Sec. 2.* The Executive Committee shall consist of the President, the Vice President, the Secretary, the Treasurer and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve out the remainder of his term.

*Sec. 3.* These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual

meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

*Sec. 4.* The officers named in Section 1 shall discharge the duties usually assigned to their respective offices.

*Sec. 5.* Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled by appointment, until the next annual meeting, by the Executive Committee.

*Sec. 6.* At the annual meeting the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than one month before the next annual meeting. It shall be the duty of the Secretary to mail the list of nominations to all members of the Society at least two weeks before the annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

#### ARTICLE IV

##### MEETINGS OF THE SOCIETY

*Section 1.* Unless previously determined by the Society the time and place of the annual meeting of the Society shall be determined by its Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

*Sec. 2.* Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

#### ARTICLE V

##### QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of its Executive Committee.

#### ARTICLE VI

##### CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society by a two-thirds vote of the members present, upon the following conditions:

(a) The proposed amendment must be in writing and signed by at least five members of the Society.

(b) This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

(c) The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

BY-LAWS

DUES

(1) The annual dues for members, unless remitted or changed by the vote of the Society, shall be seven dollars.

SECRETARY

(2) The duties and privileges of the Secretary shall be as follows:

(a) He shall keep the records of the Society.

(b) Whenever the proper officers of a number of related societies shall have a conference with a view to determining a common time and place for the several annual meetings, he shall act as the delegate or representative of this Society. (See also 5.)

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society, and

(d) He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

TREASURER

(3) The duties and privileges of the Treasurer shall be as follows:

(a) He shall be in charge of the funds of the Society.

(b) At the Annual Business Meeting of the Society he shall present a statement to date of the funds of the Society.

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society.

AUDITING COMMITTEE

(4) The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Secretary-Treasurer at the meeting for which they were appointed.

(5) The National Research Council allows the Society three representatives on the Division of Biology and Agriculture. Of these three representatives, one shall be elected each year to serve three years. The method of election shall be the same as that used in the election of the officers of the Society.

MEETINGS

(6) It shall be the policy of the Society to hold meetings in both Eastern and Central-Western territory, and the distribution of the meetings between the two territories shall be determined in general on the basis of the representation of Eastern and Western members in the Society. See also 2-c.

PROGRAM RULES

(7) In matters relating to programs for annual meetings the following rules shall be observed:

(a) Papers shall be listed and presented according to subject matter in the following groups: 1. Comparative Anatomy; 2. Embryology; 3. Cytology; 4.

Genetics; 5. Comparative and General Physiology; 6. Ecology, and 7. Miscellaneous, or other groups at the discretion of the Secretary-Treasurer.

(b) Whenever conditions require it the Executive Committee shall schedule two or more groups for the same hour and rearrange the program to bring together papers on subjects of more general interest for meetings of the whole Society. The Committee, however, is instructed to avoid conflicts as much as possible.

(c) Papers shall be listed in their respective groups in the order received. When a member offers more than one paper those following the one designated first shall be placed at the end of the list and shall not be read until all first papers by members shall have been twice called for.

(d) All papers not read when called for as listed shall be placed at the end of the group list, and, if not read when called for the second time, they shall be read by title only.

(e) The titles of "introduced" papers shall be listed in the groups after the titles of papers to be read by members. Such papers shall be read by title only in case the entire program cannot be completed during four regular sessions for reading papers.

(f) Fifteen minutes shall be the maximum time allowed for the presentation of a paper.

(g) Abstracts of papers for publication in the proceedings of the Society must be handed to the Secretary-Treasurer or his representative before final adjournment of the annual meeting.

#### HISTORICAL REVIEW

A review of the historical antecedents of the present American Society of Zoölogists will be found in *The Anatomical Record* for January, 1917. The list of officers and meeting places of the present Society found in the same place is brought up to date and reprinted here.

## LIST OF FORMER OFFICERS

## AMERICAN MORPHOLOGICAL SOCIETY

<i>President</i>	<i>Vice-President</i>	<i>Secretary-Treasurer</i>
1890—E. B. Wilson	.....	J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. S. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

*Additional Members of the Executive Committee*

1891—E. B. Wilson	1897—J. S. Kingsley
H. F. Osborn	Bashford Dean
1892—E. L. Mark	1898—C. B. Davenport
T. H. Morgan	F. R. Lillie
1893—T. H. Morgan	1899—J. P. McMurrich
C. B. Davenport	G. H. Parker
1894—E. A. Andrews	1900—F. R. Lillie
F. H. Herrick	Jacob Reighard
1895—T. H. Morgan	1901—C. F. W. McClure
S. Watase	C. W. Hargitt
1896—E. G. Conklin	1902—H. S. Jennings
William Patten	R. G. Harrison

## AMERICAN SOCIETY OF ZOÖLOGISTS

EASTERN BRANCH	<i>President</i>	CENTRAL BRANCH
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
C. B. Davenport	1907	S. A. Forbes
W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward

## LIST OF FORMER OFFICERS

EASTERN BRANCH	<i>Vice-President</i>	CENTRAL BRANCH
Jacob Reighard	1903	H. F. Nachtrieb
W. E. Castle	1904	S. J. Holmes
William Patten	1905	William A. Loey
William Patten	1906	George Lefevre
F. H. Herrick	1907	H. B. Ward
H. S. Jennings	1908	M. F. Guyer
H. V. Wilson	1909	M. F. Guyer
H. H. Wilder	1910	H. F. Nachtrieb
H. E. Crampton	1911	R. H. Walcott
G. A. Drew	1912	C. M. Child
Alex. Petrunkevitch	1913	C. M. Child

*Secretary-Treasurer*

G. A. Drew	1903	Frank Smith
G. A. Drew	1904	F. R. Lillie
H. S. Pratt	1905	C. E. McClung
H. S. Pratt	1906	T. G. Lee
C. J. Herrick	1907	T. G. Lee
L. L. Woodruff	1908	T. G. Lee
L. L. Woodruff	1909	Charles Zeleny
H. W. Rand	1910	H. V. Neal
Raymond Pearl	1911	H. V. Neal
J. H. Gerould	1912	W. C. Curtis
Caswell Grave	1913	W. C. Curtis

*Executive Committeemen*

F. R. Lillie	George Lefevre
T. H. Montgomery	T. G. Lee
H. C. Bumpus	Herbert Osborn
H. S. Jennings	C. H. Eigenmann
E. A. Andrews	J. G. Needham
W. R. Coe	S. J. Holmes
G. A. Drew	W. A. Loey
M. M. Metcalf	C. M. Child
D. H. Tennent	R. H. Walcott
R. G. Harrison	W. C. Curtis
H. E. Jordan	Oscar Riddle
C. E. McClung	H. B. Ward
	Chauncey Juday
	H. W. Norris
	C. E. McClung
	H. F. Nachtrieb

## AMERICAN SOCIETY OF ZOÖLOGISTS (AMALGAMATED)

<i>President</i>	<i>Vice-President</i>	<i>Executive Committeemen</i>
1914. C. E. McClung	M. F. Guyer	H. E. Jordan—1 year H. F. Nachtrieb—2 years H. V. Wilson—3 years George Lefevre—4 ydars A. F. Shull—5 years
1915. W. A. Locy	W. E. Ritter	D. H. Tennant
1916. D. H. Tennent	Charles Zeleny	R. P. Bigelow—5 years L. J. Cole—4 years
1917. M. M. Metcalf	Charles Zeleny	H. V. Wilson
1918. George Lefevre	L. L. Woodruff	M. M. Metcalf
1919. C. M. Child	H. H. Wilder	George Lefevre
1920. Gilman A. Drew	Caswell Grave	C. M. Child
1921. Charles A. Kofoid	Aaron L. Treadwell	G. A. Drew
1922. H. H. Wilder	Bennet M. Allen	G. A. Kofoid

*Secretary-Treasurer*

1914-1918. Caswell Grave  
1918-1921. W. C. Allee

*Secretary*

1921. W. C. Allee

*Treasurer*

1921. D. H. Tennent

## LIST OF PLACES OF MEETING

## AMERICAN MORPHOLOGICAL SOCIETY

1890—Boston	1894—Baltimore	1899—New Haven
1891—Philadelphia	1895—Philadelphia	1900—Baltimore
1892—Princeton	1896—Boston	1901—Chicago
1893—New Haven	1897—Ithaca	1902—Washington
	1898—New York	

## CENTRAL NATURALISTS

1899—Chicago                      1900—Chicago

## SOCIETY OF AMERICAN ZOÖLOGISTS

1901—Chicago                      1902—Washington

## AMERICAN SOCIETY OF ZOÖLOGISTS

<b>EASTERN BRANCH</b>	<b>JOINT MEETINGS</b>	<b>CENTRAL BRANCH</b>
1903—Philadelphia	1905—Ann Arbor	1903—St. Louis
1904—Philadelphia	1908—Baltimore	1905—(Mich.) Chicago
1906—New York	1911—Princeton	1907—(Mich.) Madison
1907—New Haven	1912—Cleveland	1907—Chicago
1909—Boston	1913—Philadelphia	1910—(Apr.) Iowa City
1910—Ithaca		1910—Minneapolis
		1912—(Apr.) Urbana

## AMERICAN SOCIETY OF ZOÖLOGISTS

## MEETING PLACES

1914—Philadelphia  
1915—Columbus  
1916—New York

1917—Minneapolis  
1918—Baltimore

1919—St. Louis  
1920—Chicago  
1921—Toronto



# AMERICAN SOCIETY OF ZOÖLOGISTS

## OFFICERS AND LIST OF MEMBERS<sup>1</sup>

### *Officers for 1922*

<i>President</i> .....	H. H. WILDER
<i>Vice-President</i> .....	BENNET M. ALLEN
<i>Secretary</i> .....	W. C. ALLEE
<i>Treasurer</i> .....	D. H. TENNENT

### *Executive Committee*

	<i>Term expires</i>
M. M. METCALF.....	1922
GEORGE LEFEVRE.....	1923
C. M. CHILD.....	1924
GILMAN A. DREW.....	1925
CHARLES A. KOFOID.....	1926

### *Representatives of the Society in the Division of Biology and Agriculture of the National Research Council*

	<i>Term expires</i>
F. R. LILLIE.....	1923
H. S. JENNINGS.....	1924
G. H. PARKER.....	1922

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<sup>1</sup> The data given in this list is based on the last preceding list published in *The Anatomical Record*, Vol. 20, No. 2, with such corrections and additions as have come to the attention of the Secretary. Please notify the Secretary of errors in this copy of the membership list that they may be corrected in the next published list.

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Resumen por los autores, Alden B. Dawson y J. H. Reis.

Una irrigación arterial anómala en la suprarrenal, riñón y ovario.

En el presente trabajo se discuten arterias anómalas halladas en un feto humano a término. En la arteria renal derecha 'normal,' que penetra en el riñón a través del hilus, nace una arteria renal accesoria, la cual después de enviar dos ramas suprarrenales pasa sobre la superficie antero-superior del riñón enviando ocho ramas a su tejido, terminando finalmente en la glándula suprarrenal. La primera rama suprarrenal se reúne con un plexo arterial, del cual nacen cuatro delgadas ramas suprarrenales. De este plexo parten también ramas que terminan en la musculatura dorsal. El tronco renal izquierdo está dividido en tres ramas. La rama inferior penetra en el riñón a través del hilus. Las ramas media y superior, las arterias renales accesorias, se distribuyen sobre el polo superior. La rama renal media envía una rama accesoria a la glándula suprarrenal. La renal superior envía dos ramas a la suprarrenal (arterias suprarrenales media e inferior), y dos ramas a la musculatura dorsal. La arteria ovárica derecha se origina dentro del riñón, estando formada por la unión de varias ramas de las arterias renales interlobares y, después de emerger a través del polo inferior del riñón, sigue un trayecto normal. Estas anomalías son interpretadas por los autores como una prueba en favor de la opinión de Bremer, quien supone la existencia de un plexo periaórtico temprano del cual derivan las arterias renal, suprarrenal y la que se distribuye sobre la glándula sexual.

Translation by José F. Nonidez  
Cornell Medical College, New York

## AN ANOMALOUS ARTERIAL SUPPLY TO SUPRARENAL, KIDNEY AND OVARY

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### TWO FIGURES

Anomalous blood vessels are always interesting from a purely scientific point of view, especially since they so often shed light on obscure problems of phylogeny and ontogeny. They may also be of considerable significance from a clinical or surgical standpoint.

Variations of the suprarenal and renal arteries occur very frequently, and anomalies of the ovarian or internal spermatic arteries are not uncommon. The cause of the extreme variability of these blood vessels is found in their embryonic history, and since they are derived from a common source (Broman, '06, '07; Bremer, '15), all combinations of the several vessels are to be expected. The anomalies in the specimen described in this paper illustrate rather nicely some phases of arterial development as outlined by Bremer ('15) in his discussion of the origin of the renal artery in mammals.

### DESCRIPTION

The subject of the present description was a well-proportioned, full-term human fetus (female). The anomalies noted were discovered during the regular dissection in the course in infant and fetal anatomy and the body was put aside for further study. Apart from the variations to be discussed in detail here, the specimen exhibited several other important arterial anomalies. There was no right innominate artery. The right subclavian artery arose as the last branch from the left extremity of the arch of the aorta and passed obliquely toward the right side,

behind the trachea, oesophagus and right carotid. The two common carotids were united into a very short single trunk. In addition to this, the right umbilical artery was absent. This last anomaly is important, since a single umbilical artery is usually associated with fusion of the lower limbs (sympodia), and it will be described elsewhere.

The inferior phrenic arteries arose from the coeliac axis. They appeared relatively large and each gave off several small branches (superior suprarenal) to the suprarenal gland of its own side. (The course of the arteries is not shown in figure 1, as the glands are pulled upward to show other details of the circulation.) The right middle suprarenal was comparatively small. In the fetus it usually approximates the renal artery in size. It arose directly from the aorta. The left middle suprarenal artery arose from an accessory renal artery.

The arterial supply to the right kidney arose from the aorta as a single trunk, but soon divided into two vessels of almost equal size. The superior division (an accessory renal artery) gave off almost immediately a small suprarenal branch, which has been designated the inferior suprarenal, although other suprarenal branches arose from this same division of the renal trunk. After giving off the inferior suprarenal branch, the accessory renal artery ran but a short distance when it was divided into two branches, forming a superior and an inferior division. The superior branch ran upward to the suprarenal gland by way of an arterial plexus and terminated in four slender branches. The arterial plexus with which it communicated sent branches also into the dorsal musculature, the right psoas major muscle and the right crus of the diaphragm. The inferior branch of the accessory renal artery also terminated in the suprarenal gland, but it passed over the anterior superior surface of the kidney and sent at least eight branches into the renal tissue. The inferior division of the main renal trunk, the normal renal artery, entered the kidney at the hilus.

The arterial supply to the left kidney also arose from the aorta as a single trunk. It soon divided, however, into three main branches, a superior, a middle, and an inferior. The



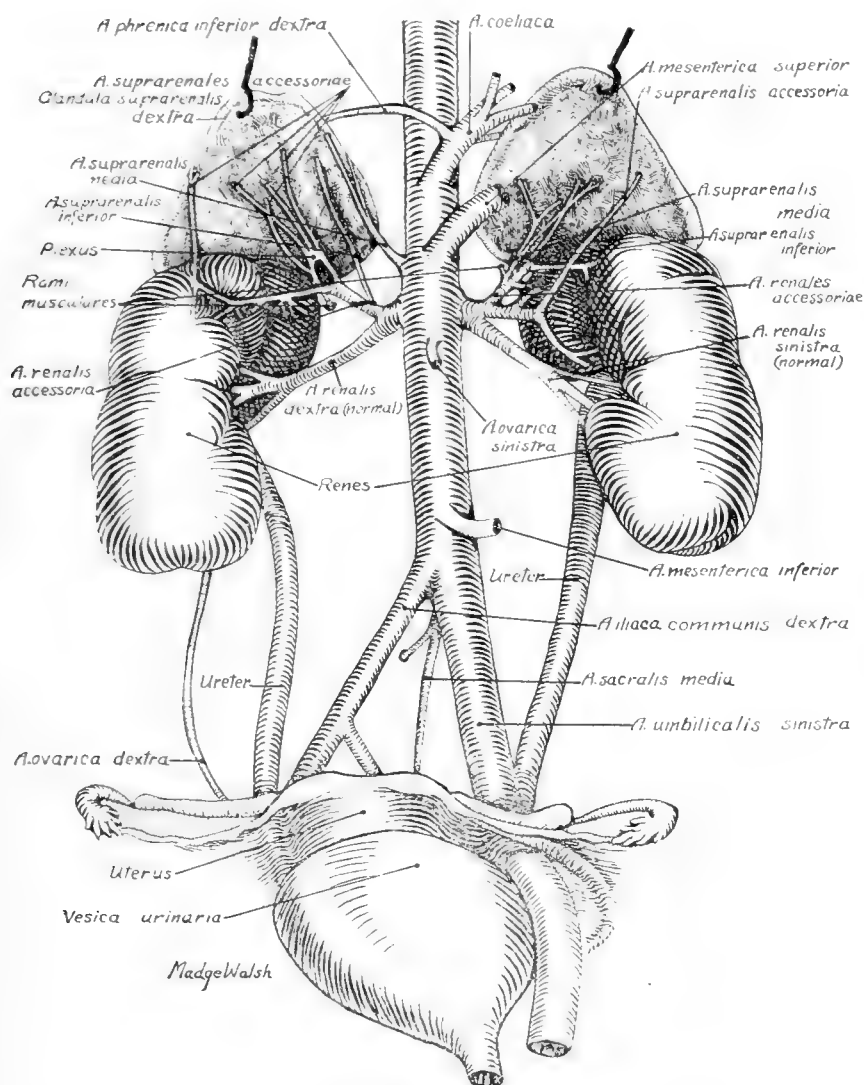


Fig. 1 Semidiagrammatic drawing of the abdominal aorta and its main branches (viewed from in front), showing the anomalous arterial supply to the suprarenal glands, kidneys, and right ovary. The suprarenal glands are pulled upward off the superior poles of the kidneys. Note the single umbilical artery (left) and the lateral displacement (to the right) of the uterus and urinary bladder.

inferior branch was the largest and entered the kidney at the hilus. It corresponds to the normal renal artery. The middle branch, an accessory renal artery, passed to the superior pole of the kidney, sent two branches into the renal tissue, and continued on to the suprarenal gland as an accessory suprarenal artery. The superior branch, also an accessory renal artery, divided irregularly into several branches. Two branches entered the kidney at its superior pole; two passed dorsally into the musculature of the body wall and two others supplied the suprarenal gland. Since, on the left side, there was no middle supra-

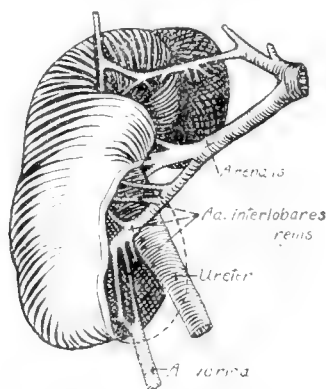


Fig. 2 The right kidney, ventral view, with the medial half of the inferior pole dissected away, to show the connections between the anomalous ovarian artery and the interlobar renal arteries.

renal arising directly from the aorta, the first of the suprarenal branches was regarded as the middle suprarenal, and in accordance with this interpretation the second branch was designated the inferior suprarenal artery.

The right ovarian artery exhibited by far the most striking variation. It arose from the renal artery, but, unlike the variations previously described in which the ovarian arteries were given off before the kidney was reached, the artery in this case was given off within the kidney, being formed by the union of three branches from the interlobar renal arteries (fig. 2). It emerged from the inferior pole of the kidney (fig. 1), and its

course and relations beyond the kidney were practically the same as for the normal artery. The left ovarian artery arose from the ventral surface of the aorta a short distance below the renal arteries.

#### EMBRYOLOGICAL SIGNIFICANCE

There is not complete unanimity regarding the embryological history of the arterial supply to the suprarenal glands, kidneys, and sex glands. Broman ('06, '07) derives the arteries supplying these organs from the mesonephric arteries. Bremer ('05), however, disagrees, partially at least, with Broman's account. According to Bremer, the arteries are developed from 'an anastomosing periaortic net having many connections with the aorta.' This net or plexus usually unites dorsal segmental, mesonephric, and ventral arteries. Bremer also points out that the history of this development differs in different mammals, being influenced in man by the lesser curvature of the rump region, the small size of the wolffian body, and the early appearance and rapid development of the suprarenal gland, the result being that in man the suprarenal gland primarily receives the vessels which in other mammals go to the kidney.

Of these numerous anastomosing arteries, only three survive ordinarily. An ascending branch from the uppermost usually becomes the inferior phrenic artery, a descending branch of the lowest becomes the normal renal artery, while the middle one persists as the middle suprarenal artery. Whether or not the normal channels are to be utilized is probably determined mechanically to some extent. If the normal course is blocked, other more devious paths may be followed.

The relations, described here of the accessory arteries to the kidneys and to the suprarenal glands, and the anastomosing connection within the kidney between the normal renal arterial supply and the ovarian artery, support Bremer's claim of an early periaortic net.

Additional evidence in favor of this view is found in three cases (IV, VI, and IX) of anomalous renal arteries described by Gillaspie, Miller, and Baskin ('16). In case IV, a left acces-

sory renal artery was given off from the superior mesenteric artery. In case VI, one of the four large-sized renal arteries supplying the right kidney arose from the coeliac axis. These cases indicate an early connection between the lateral and ventral aortic branches.

In case IX the main renal trunk on the right side was connected with the aorta by a plexus of vessels of varying sizes, and on the left side two small arteries came from a plexus which resembled the periaortic plexus. The varying arterial plexi noted can be regarded as examples of local persistence of the early anastomosing periaortic net. That these connections must be 'early' and not 'late' or secondary is shown by Bremer, since they can only be formed from vessels present in the embryo before the aorta and its larger branches develop mesodermal coats (10- to 12-mm. embryos). Towards the close of his paper Bremer ('15) states: "Dorsal connections of the suprarenal arteries with the body wall or with the true dorsal segmented arteries can be conjectured as a persistence or extension of the plexus." His conjecture is realized in the fetal circulation described, since part of the abnormal suprarenal supply was linked with a plexus which sent branches into the dorsal body wall.

#### SUMMARY

Anomalous arteries, found in a full-term human fetus, are discussed in this paper. Arising from the 'normal,' right, renal artery which entered the kidney at its hilus, there was an accessory renal artery, which, after giving off two suprarenal branches, passed over the anterior superior surface of the kidney, sent eight branches into its tissue, and terminated finally in the suprarenal gland. The first suprarenal branch was regarded as the inferior suprarenal artery. The second branch joined an arterial plexus, from which four slender suprarenal branches arose. From this plexus branches were also given off to the dorsal musculature.

The left renal trunk was divided into three branches. The inferior branch entered the kidney at the hilus. The middle and superior branches, accessory renal arteries, supplied the superior pole. The middle renal branch sent an accessory branch to

the suprarenal gland. The superior renal branch sent two branches to the suprarenal gland (middle and inferior suprarenal arteries), and two branches into the dorsal musculature.

The right ovarian artery arose within the kidney, being formed by the union of several branches from the interlobar renal arteries, and after emerging from the inferior pole of the kidney pursued a normal course.

These anomalies are interpreted as supporting Bremer's claim of an early periaortic plexus from which the renal, suprarenal, and sex-gland arteries are derived.

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Resumen por el autor, W. E. Sullivan.

### La anatomía de una mano sindáctila.

La mano estudiada presenta una unión completa de los dedos mediante membranas y una fusión parcial de los elementos óseos. Los carpianos están reducidos a seis, los metacarpianos a cuatro y las falanges a siete. Los músculos más modificados son los flexores largos; los lumbricales, que faltan; los interóseos, de los cuales hay solamente uno. Las inserciones de los flexores largos han emigrado proximalmente hasta transformarse en flexores de la muñeca. Las modificaciones musculares y óseas están relacionadas parcial, no completamente. Elementos fibrosos tienden a sustituir a los elementos óseos ausentes. La condición vascular, aunque no es la ordinaria, pudiera presentarse en una mano normal. La distribución de los nervios está relacionada directamente con otras modificaciones. La extirpación de los dos dedos intermedios aumentaría la utilidad de la mano.

Translation by José F. Nonidez  
Cornell Medical College, New York

## THE ANATOMY OF A SYNDACTYLOUS HAND

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ONE FIGURE

The hand in question may be referred to as brachydactylous as well as syndactylous, for there is a reduction of one phalanx in each digit in addition to the webbing and fusion. It is the right hand of a male laborer about forty years old. The left hand is normal. Nothing is known of the family history.

The general shape of the hand is sufficiently indicated by the radiograph. The volar surface is unbroken and there is no indication of separate digits. The thenar and hypothenar eminences are well marked. The thenar eminence is slightly reduced, but the hypothenar would equal the average normal elevation. The skin is thick, creased, and calloused and indicates a serviceable hand.

On the dorsal surface the presence of separate digits is suggested by three well-defined nails. These three nails are fairly indicative of the internal condition. The x-ray shows part of a fourth digit and suggests that there are five terminal phalanges. Dissection shows more clearly that there are five terminal phalanges.

In the discussion it seems best to refer to the digits as the radial, radial intermediate, ulnar intermediate, and ulnar in the order indicated. From the relations found on dissection it was interpreted that the radial digit represents the first and second digits, the radial intermediate, the ulnar intermediate, and the ulnar represent the third, fourth, and fifth digits, respectively.

## MODIFICATION OF THE BONES

The bones of the forearm show little if any modification. The distal end of the radius is slightly enlarged.

Six carpal elements are present. These are interpreted as representing the ossa naviculare and lunatum in the proximal row, the multangulum majus, capitatum, pisiforme, and triquetrohamatum in the distal row. The position and articulations of the several bones are seen in the radiograph. It may be pointed out that the distal end of the navicular overlaps the volar surface of the greater multangular. The os multangulum minus is absent. The os capitatum is greatly modified and corresponds to the proximal portion of the normal bone. The condition of the last two bones is in line with the general modification of the second and third digits. The os pisiforme is much flattened and somewhat larger than is usual. The os triquetrohamatum is the name given to the large medial bone of the distal row. Its position and articulations as well as its size and shape indicate that it is the result of the fusion of the triquetrum and hamatum. The hamulus is much reduced.

The metacarpals show a considerable range of modification. The radial metacarpal is normal at its proximal end, but at the distal end presents two large articular surfaces, somewhat separated by a ridge, for the phalanges, and a small volar facet for a sesamoid.

The metacarpal of the radial intermediate digit is represented proximally by a small, ovoid, bony element. This bony element is imbedded in a wedge of fibrous tissue which has its base against the capitate and which, at its distal end, is connected with the phalanx by a round ligament more than a millimeter in diameter.

The two medial metacarpals are fused at their proximal ends. At the distal end each presents an articular surface for its phalanx.

In addition to the loss of one phalanx in each digit, there are one or two interesting modifications. The radial phalanx is large and irregular and is formed by the fusion of two terminal phalanges. The proximal end has two distinct articular surfaces, well separated by a groove. The more medial lies some-



what dorsad. On the dorsal surface the individuality of the two phalanges is clearly seen. The fact that the radial digit is equal in length to the other digits naturally suggests that there has been a fusion of the proximal and distal phalanges rather than the loss of a phalanx. There is nothing in the structure of the phalanx to indicate that this has been the case.

The proximal phalanx of the radial intermediate digit represents only the distal end of a phalanx, but, as already pointed out, it is joined to the metacarpal by a heavy ligament. The proximal phalanx of the ulnar intermediate digit is fused with its metacarpal and is somewhat longer than the other phalanges.

#### MODIFICATION OF THE MUSCLES

The forearm is well developed and at the elbow is but slightly smaller than its fellow. The muscles inserting on the radius and ulna are not modified. Those having a more distal insertion may be best discussed individually.

The palmaris longus, palmaris brevis, and flexor carpi ulnaris are normal.

The thenar muscles as a group are fairly well developed and arise from the lateral half of the transverse carpal ligament. The deeper fibers insert into the metacarpal. The more superficial fibers separate into two bundles to insert in part mediad and in part laterad on the volar surface of the base of the phalanx. The innervation suggests the absence of the adductor pollicis.

The hypothenar muscles are better developed than the thenar group. They arise from the pisiforme and medial half of the transverse carpal ligament and insert into the metacarpal and proximal phalanx. All of the hypothenar muscles are present.

But one interosseous muscle is present. It is a poorly developed volar interosseous of the ulnar digit.

The flexor digitorum sublimis is relatively small and ends in two small tendons which insert into the capitata and hamate. In addition, the tendons of the sublimis and profundus converge to form a common tendon of insertion into the distal row of carpals and the bases of the metacarpals. It acts only as a flexor of the wrist-joint.

The flexor digitorum profundus is of normal size. It goes over for the most part into the common tendon with the sublimis, but sends one tendon to the proximal phalanx of the ulnar digit. This tendon gives flexion and adduction at the metacarpophalangeal joint. The muscle as a whole acts as a flexor of the wrist-joint.

The lumbricals are absent.

The flexor carpi radialis inserts into the radial metacarpal.

The extensor carpi radialis longus inserts on the dorsum of the base of the radial metacarpal. The extensor carpi radialis brevis is absent.

The extensor digitorum communis has two tendons of insertion. The lateral tendon inserts on the phalanx of the radial digit. The medial tendon lies in the plane of the ulnar intermediate digit and for the most part inserts into the metacarpal. In addition it sends a slip to the radial intermediate metacarpal and a larger slip which attaches rather loosely to the dorsum of the ulnar metacarpal.

The abductor pollicis longus inserts into the lateral surface of the base of the radial metacarpal. Just dorsal to this is the insertion of the extensor pollicis brevis.

The extensor pollicis longus has an origin corresponding to the combined origin of itself and the extensor indicis proprius. It inserts into the terminal phalanx of the radial digit well toward the lateral margin.

The extensor digiti quinti proprius inserts into the proximal phalanx of the ulnar digit.

#### VARIATIONS IN THE ARTERIES

The ulnar and interossei vessels supply the forearm and hand. The radial artery is absent, while the volar interosseous is much enlarged. Such a condition might occur in an otherwise normal hand, and it is perhaps unwise to correlate it with the other factors.

There is but one volar arch which is formed largely by the ulnar, but which is completed by a perforating branch from the volar interosseous. The terminal branches of the volar interosseous supply the dorsum of the wrist and hand.

#### NERVES

The cutaneous distribution of the nerves showed nothing beyond the usual range of variation. On the dorsum of the hand they may be traced farther distally on the terminal phalanx than is usually possible on a normal hand.

#### MOVEMENTS AT THE SEVERAL JOINTS

The radiocarpal and carpometacarpal are biaxial joints. The movements in the former are normal. In the latter flexion and extension are possible through a range of about 80°, but abduction and adduction are modified in several ways. The fusion of the bones in the two medial digits as well as the general webbing restrict the movements. Splitting the hand and removing all of the ulnar intermediate digit except the base of the metacarpal added greatly to the range of forced movement.

#### SUMMARY

Muscular and osseous modifications are partially but not completely correlated. Fibrous elements tend to take the place of absent bony elements.

The vascular condition, while unusual, might occur in a normal hand.

The distribution of the nerves is directly correlated with the modification of the other structures.

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## PLATE 1

### EXPLANATION OF FIGURE

Radiograph of a right hand. In the text the digits are referred to as radial, radial intermediate, ulnar intermediate, and ulnar.



Resumen por el autor, Warren H. Lewis.

### ¿Es el mesenquima un sincicio?

Una revisión de la literatura referente al mesenquima considerado como un sincicio no ha revelado una sola prueba sobre la fusión de los procesos celulares, produciendo continuidad citoplásmica. En material fijado es imposible decidir si se trata de una adhesión o de una fusión. Los crecimientos del mesenquima embrionario en cultivos de tejidos procedentes de embriones de pollo forman un retículo semejante al que se observa en el embrión. Puede seguirse la lenta emigración de las células, la retracción de los procesos de las células vecinas y la producción de nuevos procesos en la misma o en otras células. Las células con muchos procesos pueden aislarse completamente de todas las demás células del retículo y, sin embargo, no se comportan de un modo diferente del de las células que forman aquel. Los procesos adheridos al cubre objetos se comportan lo mismo que los que se insertan en células vecinas, con la excepción de que los primeros se adhieren más firmemente. No existe prueba alguna sobre la transferencia de material alguno desde unas células a otras. Si se añaden soluciones hipertónicas a los cultivos, las células se transforman en redondas, perdiendo toda conexión con las células vecinas. En un tipo de degeneración se produce un efecto algo semejante. Las células hijas no permanecen fusionadas de un modo permanente sino que finalmente se encuentran en la misma relación que las demás células del retículo, o hasta pueden perder toda conexión. Las pruebas obtenidas mediante el estudio de los cultivos indica que el mesenquima embrionario es un retículo adherente y no un sincicio.

Translation by José F. Nonidez  
Cornell Medical College, New York

## IS MESENCHYME A SYNCYTIIUM?

WARREN H. LEWIS

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FOUR FIGURES

Many anatomists consider the mesenchyme a syncytium. They believe there is actual fusion of process with process, continuity of the cytoplasm, and absence of cell boundaries. In ordinary preparations of embryonic and adult material such fusion of cell processes appears to exist; but is this sufficient evidence upon which to base a distinction between actual fusion and mere adhesion? If one stops to consider the extreme delicacy of such processes in the embryonic mesenchyme, one must admit that here, at least, the methods used have not been adequate to settle the question. In fixed material it is simply impossible to tell whether there is fusion or adhesion. A review of the literature dealing with mesenchyme as a syncytium has not disclosed to me one single instance of actual proof that mesenchyme really is syncytial in nature. It is a question which cannot be solved by the methods now employed. The idea that mesenchyme cells are fused together dates back at least as far as the time of Max Schultze ('61),<sup>1</sup> and from that time to the present this view seems to have prevailed among the majority of investigators who have studied the origin and development of the connective tissues. No special effort has been made in the past to prove this particular point, for the reason, I suppose, that ordinary sections appeared to show clearly that the cells were fused, and so it seemed too much a matter of fact to call for a special investigation.

In the early days of tissue culture we used the term 'syncytium,' adopting the prevailing view of the nature of this tissue,

<sup>1</sup> Schultze, Max. 1861 Ueber Muskelkörperchen und das, was man eine Zelle zu nennen habe. *Archiv. f. Anat. und Physiol. Reichert.*

since the outgrowths resemble in general character the tissue as it exists in the embryo. From the behavior of such cells in tissue cultures, however, I have gradually come to doubt the correctness of the view that the mesenchyme is syncytial in nature, and it is my purpose here to present briefly the evidence.

The cultures utilized in the study were all from embryonic chick material of various organs and regions, for the most part from the subcutaneous tissue. They were made in the usual manner with fluid media consisting of Locke's solution 80 parts, dextrose 0.25 to 0.5 per cent, and chicken bouillon 20 parts.

The general appearance of the mesenchymal reticulum in the more normal cultures is strikingly similar to that in the embryo, and there is no reason to believe that the nature of the attachment of one cell to another is different in the cultures from that in the embryo.

The first cells that migrate out often become entirely isolated from the explant and from one another, but as the number increases they crowd together, and a complicated reticulum, similar to that in the explant, is formed in the outgrowth. Thus it would seem that the early migrating cells have no difficulty in becoming entirely isolated from the reticular network in the explant of which they were a part, by forces which pull or force them out.

It has been assumed by many that the mesenchymal syncytium was brought about in part, at least, by the failure of the cytoplasm to divide completely after division of the nucleus. We have often observed a lingering connection between daughter cells for an hour or more after division. Many daughter cells, however, ultimately move some distance apart and the character of their attachment is then no different from that between these and neighboring cells.

In living cultures in fluid media the outgrowths of mesenchyme, as well as of most other types of cells, tend to be forced by the conditions of the environment into a single plane lying between the cover-glass and the fluid. The cell bodies, of course, project down into the fluid, but this becomes less and less marked as the cells flatten out more and more towards the



periphery of the outgrowth. Near the explant the cells are often more than one layer thick and their behavior, therefore, cannot be successfully observed; but the conditions at and near the periphery, or even in the middle of the outgrowth, afford an unusual opportunity for examining the relationship between the processes of one cell and the processes and bodies of neighboring cells (figs. 1 and 2). At the periphery the cells not only are more flattened, but usually become more widely separated and even entirely isolated from neighboring cells. In this region one can watch the slow shifting of cells from one position to another and can follow the withdrawal of processes which were adherent or in contact with the processes or bodies of neighboring cells. There is no reason to believe that such processes were previously fused with the processes or bodies of the neighboring cells, even though it is usually impossible to determine this by observation. In the slow withdrawal of the processes there is usually no evidence of a rupture. Many of the processes end on the cover-glass; except for the fact that they are more firmly adherent, there is apparently no difference in character between these processes and those attached to neighboring cells, and their withdrawal or change of shape proceeds in a similar manner. Simultaneously to the withdrawal of processes from neighboring cells or from the cover-glass, new processes are sent out along the cover-glass to the same cells or other cells and to other positions on the cover-glass. As cells become separated more and more at the periphery they may lose all connection with neighboring cells, their processes extending out and ending on the cover-glass. Such cells behave no differently from those forming part of the reticulum. This shifting of the relative positions of cells and of the area of attachment of their processes, and the changes in the size and shape of these areas of attachment, all indicate adhesion rather than fusion.

It is not uncommon, in cultures that have just been washed with a new solution of one kind or another, to find that the mesenchyme cells are drawing in their processes and losing connection with the neighboring cells and with the cover-glass. Such contraction may proceed until all connections with neigh-

boring cells are lost; it may even be so extreme that the cells lose their adhesion to the cover-glass, when the rounded cells may fall from the under-surface of the cover-glass to the lower surface of the drop. If, however, the cells retain their attachment to the cover-glass, they may later send out new processes onto the cover-glass, some of which may find their way to neighboring cells and their processes, and a reticulum is again established. Sometimes contraction is so violent that here and there processes are broken, the peripheral end remaining attached to a neighboring cell or the cover-glass, the proximal part withdrawing into the cell. Sometimes the mere transfer of the slide from the incubator to the warm observation box is sufficient to produce withdrawal of processes and more or less rounding up of the cells.

Hogue ('19)<sup>2</sup> found that when cultures of mesenchyme cells were treated with hypertonic Locke-Lewis solution, many of them withdrew their processes, became irregularly rounded, and lost connection with other cells, since all processes disappeared. Mrs. Lewis ('20)<sup>3</sup> found that in cultures of smooth muscle a minute amount of glycerin introduced into the neighborhood of the growth caused all cell processes to be immediately withdrawn, so that the cells became isolated individuals and remained so for several hours after the abnormal environment had been removed.

We should hardly expect cells to behave in this manner if they were actually fused together, though of course one cannot deny the possibility that they might do so, even under that condition. The mode of withdrawal of the processes in the rounding up of cells, however, indicates adhesion. When processes withdraw into the cell body, the line of separation does not come in the intermediate area between the bodies, but the processes seem to slip off from each other or from the cell bodies, just as they do when they are withdrawn under more normal conditions.

<sup>2</sup> Hogue, M. J. 1919 The effect of hypotonic and hypertonic solutions on fibroblasts of the embryonic chick heart in vitro. *J. Exper. Med.*, vol. 30.

<sup>3</sup> Lewis, M. R. 1920 Muscular contraction in tissue cultures. *Contributions to Embryology*, vol. 9. Carnegie Inst. Wash., Pub. 272.

In one type of degeneration the processes are gradually withdrawn into the bodies of the cells and connections with neighboring cells disappear one after another until finally all connections are lost and many of the cells become entirely isolated from their neighbors. At the same time most of the processes which are attached to the cover-glass only contract into the body of the cell, and the latter assumes a more or less compact rounded shape (fig. 4). Such rounded cells may remain alive for some time and, if degeneration has not proceeded too far, a renewal of the medium may be followed by the sending out of new processes onto the cover-glass and to neighboring cells.

In fixed cultures, as in sectioned material, the network of processes in most places is so complicated that one cannot determine in the majority of cases whether the anastomoses are accompanied by fusion or by mere adhesion (figs. 1 and 2). Near the periphery, however, it is often possible to follow the outline of some very thin processes onto neighboring cells or processes of those cells, and it is not unusual for larger and longer processes to be followed over several neighboring cells (figs. 1 and 2). One cannot tell, of course, whether there is any fusion where such processes lie flat against other processes or cells in this fixed material, but the fact that under favorable conditions they appear to retain their individuality and their outlines can often be followed, in part or in their entirety, on neighboring cells, speaks against fusion. The difficulty in determining in all cases whether we are dealing with adhesion or fusion can readily be understood when we picture the conditions involved were an isolated cell such as that shown in figure 3 to be placed in such a complex network or reticulum as that shown in figure 1.

One interesting thing about the mesenchyme cells is the fact that they form in cultures a reticulum very similar to that in the embryo. Why do they behave thus and what are the factors that determine it? Why does the mesenchyme form a reticulum and the epithelium or endoderm a sheet or membrane? In the first place, we may safely conclude that the surface of the cells is sticky for each other and for the cover-glass, and that the physical factors of cohesion and surface tension or capillary attraction are constantly at work in altering the form

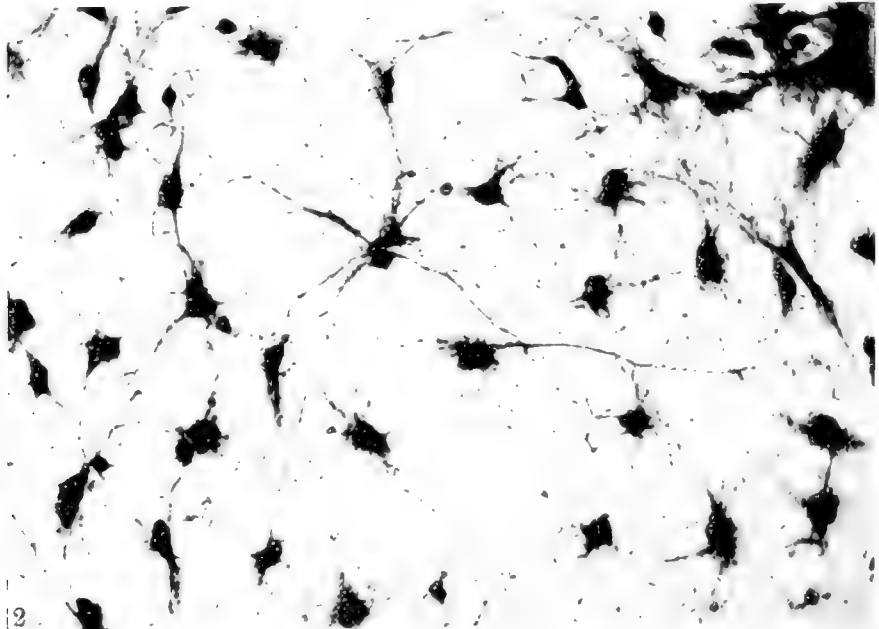
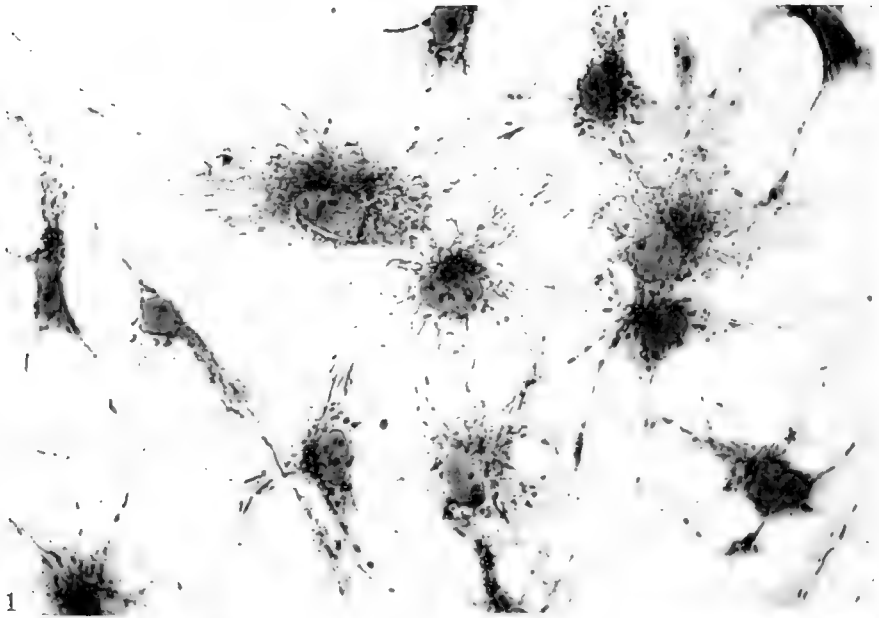
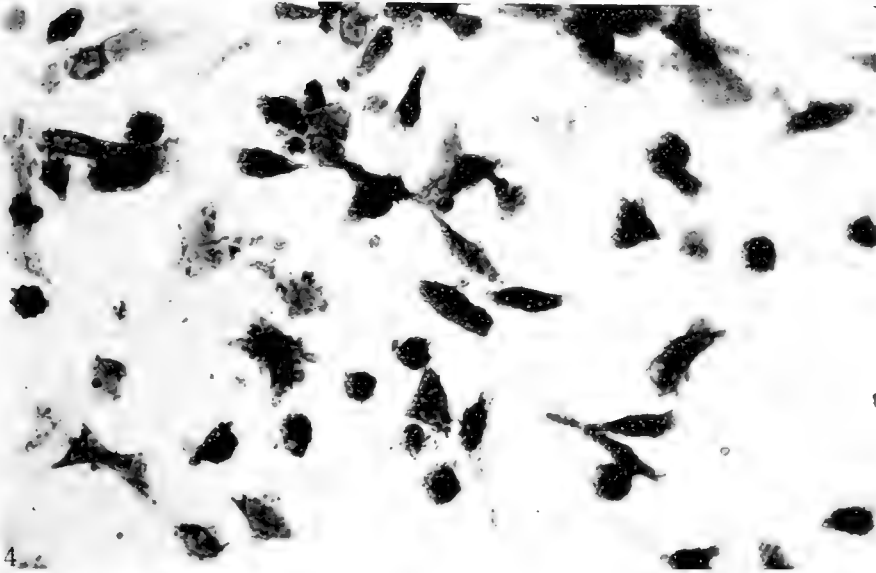


Fig. 1 Mesenchymal reticulum or network from the middle portion of the outgrowth of subcutaneous tissue from an eight-day chick embryo; two-day culture in Locke-Lewis solution; janus green, iodine.  $\times 480$

Fig. 2 Mesenchymal reticulum of subcutaneous tissue from an eight-day chick embryo; four-day culture in Locke-Lewis solution; janus green, iodine.  $\times 480$ .

3



4

Fig. 3 Isolated mesenchymal cell near the edge of the outgrowth from the same culture shown in figure 1.  $\times 1450$ .

Fig. 4 Contracting mesenchymal cells in a degenerating culture from the subcutaneous tissue of an eight-day chick embryo; four-day culture in Locke-Lewis solution; janus green, iodine.  $\times 480$ .

and position of the cells and their processes. Evidently, the cohesiveness of the cytoplasm is constantly undergoing alteration in various parts of the cell and is probably initiated by local variations in the metabolism which produce local changes in fluidity and in surface tension (Loeb, '20).<sup>4</sup> The surface tension pull of the fluid medium bathing the cells is a constant factor and acts on the changing cells to produce shifting and variation in their protoplasmic processes. If we adopt such a tentative explanation for the mesenchyme cells and attempt to explain in a similar manner the smoother edges of the ectodermal and endodermal cells, it is obvious that we must assume that the cytoplasm of the latter is not subject to such extreme local variations in cohesiveness and surface tension. We know so little about the metabolism and the physical and chemical properties of the mesenchyme that it scarcely makes any difference whether we regard the mesenchyme as a syncytium or as an adherent reticulum. Yet the fact that such cells, both in cultures and in the normal embryo, retain their individuality, no two of them being exactly alike in size and shape, in number, or arrangement of their processes, in number, size, or arrangement of the mitochondria, in number, size, or position of the granules and vacuoles, or in the size and position of the nucleus and nucleolus, speaks for adhesion and for the independence of each cell. There is no indication of the transfer of material from one cell to another. We did believe at one time that mitochondria passed from one cell to another, but more critical examination of living cultures has convinced me that this is not true.

The development of the collogenic fibers, whether their origin be intercellular or intracellular, can be explained as readily from an adherent reticulum as from a syncytium.

#### CONCLUSION

There is no evidence that embryonic mesenchyme is syncytial in structure. The evidence from tissue cultures points to the view that it is an adherent reticulum or network, and not a syncytium.

<sup>4</sup> Loeb, Leo. 1920 The movements of the amoebocytes and the experimental production of amoebocyte (cell-fibrin) tissue. Washington University Studies, vol. 8, Science Series no. 1.



Resumen por el autor, Sidney M. Cone.

Terminaciones de los nervios seccionados.

El presente trabajo comienza con una referencia al trabajo experimental de Cajal sobre la regeneración de los extremos de los nervios. El autor describe sus observaciones en el caso de nervios lesionados en heridas de guerra, en los cuales los corpúsculos de Pacini se formaron en el tejido subcutáneo de un muñón doloroso en un dedo amputado. Estos cuerpos han podido seguirse en su origen hasta un fascículo de nervios del callo nervioso vecino. El autor describe el corpúsculo de Pacini y su formación. También incluye una referencia al método de teñido.

Translation by José F. Nonidez  
Cornell Medical College, New York



## ENDINGS OF CUT NERVES

SYDNEY M. CONE

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ONE PLATE (FOUR FIGURES)

Cajal's recent article in the July number of the *Siglo Medico*, Madrid, describing the regeneration of nerve ends in experiments on animals, reports inability to obtain regeneration of end bodies of sensory nerves. The wild growth of embryonal nerves is noted, but he has not been able to demonstrate specialized end organs.

Among the many peculiar manifestations of new nerve growth following war injuries, in my collection, is one of pacinian bodies (Vater's corpuscles) in a finger stump. The ring-finger had been amputated, and a year later the stump was again removed on account of pain. The note made by me at the time of the microscopic examination calls attention to nerve callus following the nerve trunk and spreading in the subcutaneous tissue and scar. Numerous pacinian bodies were noted. The nerve callus does not differ from that which I found in all cases of war-injured nerves. The individual fasciculi, consisting of ten to twenty nerve fibers measuring 2 to 4  $\mu$  each, intertwine about the original nerve, passing beyond it to the loose subcutaneous tissue and into the stump scar. A few fasciculi break up into individual fibers at the formation of large, oval, lamellated bodies, varying in size from 60 to 150  $\mu$  long and 40 to 60  $\mu$  wide. Several capillaries of the fasciculus pass with the individual nerve fibers into the pacinian corpuscle and spread at the periphery. The nuclei of the Schwann sheath cells become even more than ordinarily elongated and flattened at the circumference, but three or four entire cells are demonstrated in the central core. The fasciculi, as they end at these bodies, are made up of elongated nuclei and varicose fibers (Peroncito's spirals). The

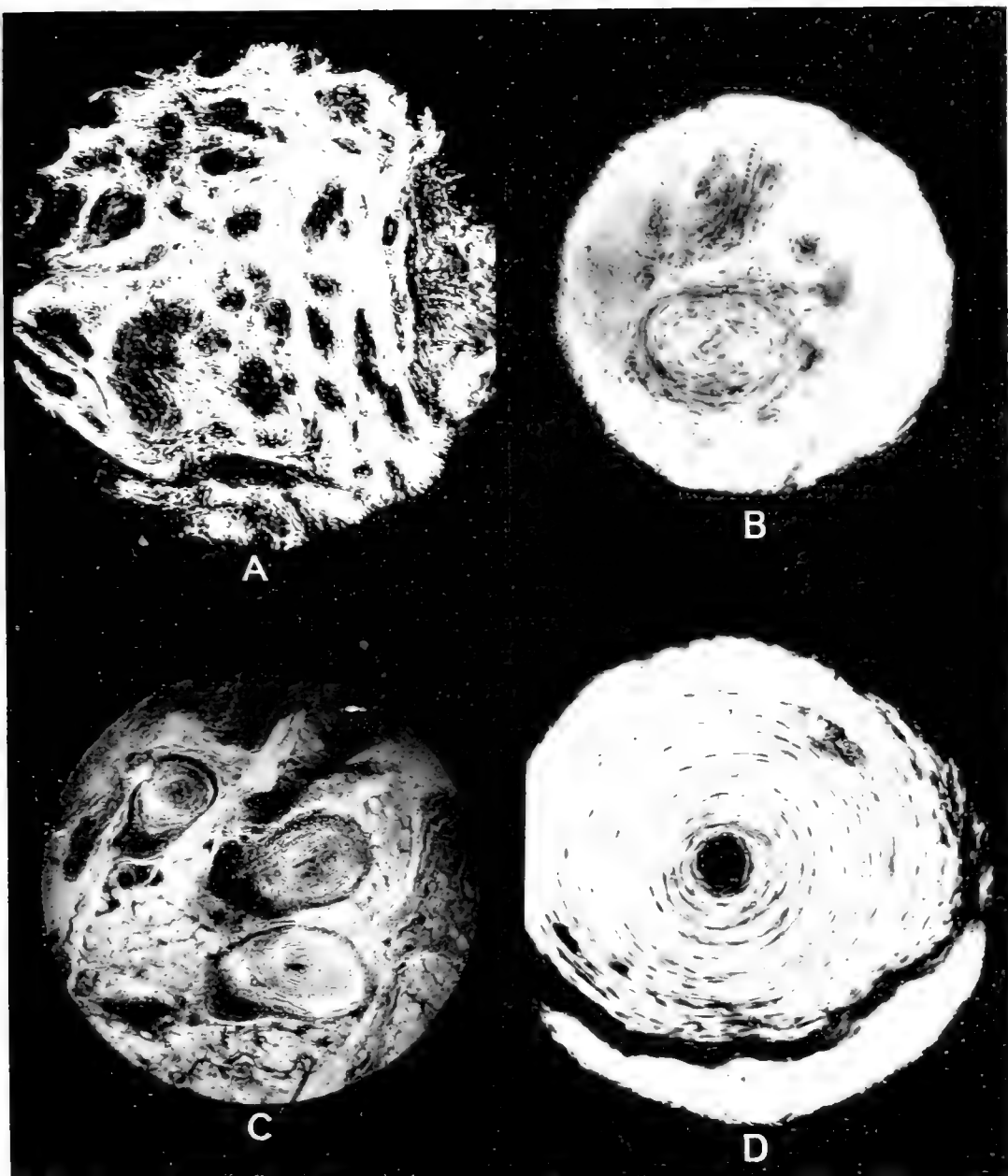
capillary vessels within the fasciculus pass into the body and also course round about the corpuscle. The nuclei are numerous and compressed between the flattened fibers. Indeed, one cannot tell the difference between the nucleus and axis-cylinder in these bodies any more than was it possible to distinguish nucleus and nerve in the embryonal fiber in nerve callus. The periphery of the body is made of thinner, more compressed layers than the center. A capsule of vascular connective tissue encapsulates the Vater corpuscle. In the center the Schwann sheath cell nuclei are swollen and well stained. There is no adult connective tissue in the bodies. The entire process looks like a swelling and rounding off of the new nerve callus tips in subcutaneous fat. These specimens were stained with the neurokeratin stain, described by me in the *British Journal of Surgery*, May, 1918.

I am greatly indebted to Prof. Hugh R. Spencer for the photographs.

#### PLATE 1

##### EXPLANATION OF FIGURES

- 1 Nerve callus about the cut digital nerve.
- 2 Fasciculus of nerve callus passing into the pacinian corpuscle.
- 3 Three pacinian bodies in subcutaneous fat.
- 4 Individual pacinian (Vater) corpuscle demonstrating stratified layers, capillaries and Schwann sheath cell nuclei.



Resumen por el autor, A. L. Salazar.

Sobre la existencia de falsos cuerpos amarillos autónomos en la glándula intersticial de la coneja.

El autor llama la atención acerca de la existencia, en el ovario del conejo (ovario de tipo intersticial), de cuerpos amarillos atréticos, con una evolución autónoma; estos cuerpos atréticos a diferencia de los ordinarios, no se amalgaman en la glándula intersticial, sino que pasan autonómicamente a través de su evolución propia, aislados dentro de una cápsula de tejido conectivo. El autor describe brevemente las características de los cuerpos atréticos autónomos, y admite la posibilidad de su confusión con viejos cuerpos amarillos genuinos, siendo probablemente esta confusión una de las causas de las opiniones contradictorias formuladas en relación con la regresión de los cuerpos amarillos verdaderos.

Translation by José F. Nonidez  
Cornell Medical College, New York

## SUR L'EXISTENCE DE FAUX CORPS JAUNES AUTONOMES DANS LA GLANDE INTERSTITIELLE DE LA LAPINE

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ONE FIGURE

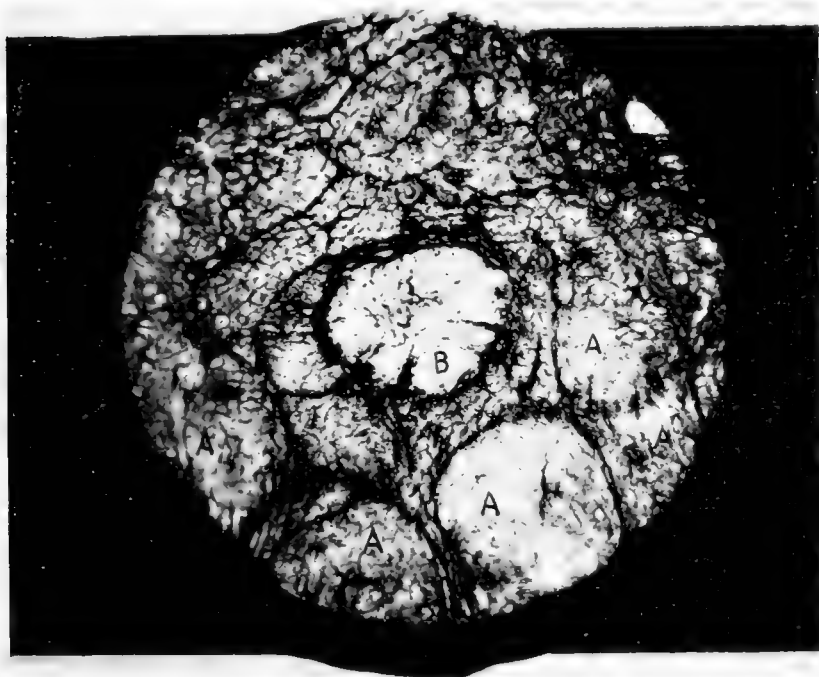
Limon, dans son travail<sup>1</sup> sur la glande interstitielle, dit que chez la Lapine "à la longue le faux corps jaune perd son individualité. Lorsqu'il ne reste plus de trace de la membrane de Slavjanski, il perd sa disposition arrondie. Il s'étire, s'aplatit transversamment." Cette description n'est pas absolument exacte; car, si quelques corps jaunes atrétiques s'aplatissent, s'étirent, au moment de leur fusionnement, d'autres conservent leur aspect arrondi: cela dépend des dimensions du follicule originaire, de leur position et d'autres circonstances, dont nous occuperons plus tard à propos de la formation du faux corps jaune. Ce que nous voulons signaler ici est que si la plupart des corps jaunes atrétiques perdent leur individualité et se fusionnent, quelques-uns conservent leur individualité pendant leur cycle, sans jamais se fusionner dans la nappe interstitielle. Nous les appellerons 'faux corps jaunes autonomes' pour les différencier de ceux qui se fusionnent dans la nappe interstitielle. Dans les coupes au tannin-fer les faux corps jaunes autonomes se reconnaissent facilement, même à de petits grossissements, grâce aux caractères suivants. Dès le moment de leur formation par atrésie folliculaire et hypertrophie de la thèque, ils s'entourent d'une épaisse coque conjonctive plus puissante que celle des faux corps jaunes habituels. Cette coque conjonctive accompagne le corps jaune atrétique autonome jusqu'au moment de sa

<sup>1</sup> Limon, Étude histologique et histogénique de la glande interstitielle de l'ovaire. Arch. Anat. Micr., T. 5, 1902.

disparition, tandis que la coque conjonctive des autres faux corps jaunes disparaît en partie au moment de leur fusion. Un autre caractère important des corps jaunes atrétiques autonomes est que la métamorphose adipeuse de leurs cellules débute dans le centre et de là irradie vers la périphérie. Il arrive un moment où toutes les cellules du faux corps jaune autonome se trouvent chargées de grosses boules lipoides. On voit alors le faux corps jaune autonome envahi par un tissu conjonctif puissant; cette invasion est due à l'effet convergent d'un envahissement conjonctif exogène et d'une prolifération endogène. Le conjonctif exogène vient de la coque conjonctive, accompagné de vaisseaux, qui en général se disposent en couronne dans la périphérie du corps jaune atrétique; le conjonctif endogène provient de la prolifération de la cicatrice conjonctive du follicule atrésié. De cette invasion conjonctive conjuguée avec la prolifération endogène il résulte la transformation du faux corps jaune dans un nodule cicatriciel. Cette transformation se fait grâce à un processus spécial, où le rôle actif appartient au conjonctif. En effet, on voit les fibres conjonctives énucléer les grosses boules lipoides qui font hernie dans les cellules. Les boules lipoides tombent ainsi dans le conjonctif qu'on trouve entièrement chargé de sphères lipoides. Certaines fibrilles conjonctives semblent être formées par les cellules interstitielles; ces faits sont à rapprocher de ceux que Mulon<sup>2</sup> a décrits dans le vrai corps jaune. Nous ne pouvons entrer ici dans les détails sur ce processus énucléateur. Les cellules présentent, dans leur évolution au dedans du faux corps jaune autonome, les mêmes caractéristiques générales que dans la nappe interstitielle: la lipogenèse s'y fait aux dépens du chondriome tannophile de la même manière. On y voit aussi de nombreux cristalloïdes. Il y a même des faux corps jaunes autonomes où prédomine la graisse, d'autres où prédominent les cristalloïdes. D'autres, enfin, sont caractérisés par la présence dans presque toutes les cellules d'un système de canalicules et lacunes étoilées. Nous ne savons rien sur la signification de ces différences; elles peuvent représenter diffé-

<sup>2</sup> Mulon, Sur un corps jaune kystique formé au dépens d'un ovisac non déhiscé. Arch. Anat. Micr., T. 11, 909, 910.

rentes étapes d'une même évolution cellulaire, ou des modalités structurales et fonctionales diverses. Nous n'avons pu non plus, jusqu'ici, observer nettement la destinée finale des cellules des faux corps jaunes autonomes; les faits observés par nous à ce propos sont assez contradictoires. Au moment de l'énucléa-



Ovaire de Lapine du type interstitiel cloisonné. A, A, A, corps jaunes atrétiques habituels; B, corps jaune atrétique à évolution autonome. Fixation, liquide de Bouin; méthode tanno-ferrique.

tion conjonctive des boules lipoïdes on voit entre les fibres collagènes des lambeaux déchirés de protoplasme interstitiel avec son aspect réticulé caractéristique; parfois on voit ici et là, dans les vieux corps jaunes autonomes des flots de petites cellules polyédriques très pauvres en protoplasme, ce qui semble indiquer que les cellules, après l'énucléation des boules lipoïdes, rentrent au repos. Mais ces faits ne possèdent pas la généralisation suffisante pour établir nettement la question. Les faux corps

jaunes autonomes sont habituellement ronds, les périphériques sont piriformes; après la prolifération conjonctive ces derniers semblent suspendus de l'albuginée par un puissant pédicule conjonctif. Nous avons observé un cas où ce pédicule était parcouru par un cordon épithélial qui naissait dans l'épithélium germinatif sous la forme d'une invagination, qui ensuite se transformait dans un cordon et pénétrait ainsi dans le pédicule conjonctif, où il se perdait. Certains corps jaunes atrétiques autonomes possèdent une coque conjonctive incomplète, en forme de croissant.

Il est bien possible qu'on ait confondu jusqu'ici les faux corps jaune autonomes avec les reliquats des vrais corps jaunes. Cette confusion peut expliquer jusqu'à un certain point les opinions si contradictoires qu'on a émises à ce propos. Nous ne pouvons cependant être affirmatifs à ce propos, car, presque toujours les travaux sont très pauvres en documents iconographiques sur les reliquats des vrais corps jaunes. Cependant, quand on étudie la littérature, on voit que les auteurs se partagent, à propos de la destinée du vrai corps jaune, en deux catégories absolument irréductibles. Pour les uns, les vrais corps jaunes participent dans la formation de la glande interstitielle, totalement,<sup>3</sup> ou partiellement,<sup>4</sup> pour les autres il subit une régression adipeuse et fibreuse. Or, les auteurs de la deuxième catégorie décrivent la régression de vrai corps jaune de telle manière, que leur description pourrait s'appliquer presque entièrement à l'évolution du corps jaune atrétique autonome. Ont-ils décrit, non des reliquats du vrai corps jaune, mais de vieux corps jaunes atrétiques autonomes? Nous l'ignorons, mais cela est probable. Cependant, on peut admettre une autre hypothèse, à savoir, qu'à partir d'un certain moment, le vrai corps jaune et le corps atrétique autonome présentent des caractères communs; seule, alors une étude minutieuse permettra de chercher les caractères différentiels. Conclusion: l'existence de corps jaunes atrétiques autonomes nous oblige à reprendre le problème, si obscur encore, de la destinée du vrai corps jaune.

<sup>3</sup> Pflüger et V. Ebner.

<sup>4</sup> V. der Stricht, etc.



Les faux corps jaunes autonomes n'existent pas dans tous les types d'ovaire; ils se montrent dans les ovaires du type interstitiel.<sup>5</sup> Mais tous les ovaires de ce type ne présentent pas des corps atrétiques autonomes en nombre égal. Certains ovaires à type interstitiel non cloisonné,<sup>6</sup> très volumineux, ayant dans la nappe interstitielle des champs de cellules interstitielles à type lutéinique, présentent de rares corps autonomes ou n'en présentent aucun; d'autres ovaires du type interstitiel cloisonné présentent de nombreux corps atrétiques autonomes. Ils semblent, donc, caractériser un moment donné de l'évolution de l'ovaire; mais cette question exige de plus amples recherches.

Jusqu'ici nous n'avons pas trouvé des différences cytologiques entre les cellules qui évoluent dans la nappe interstitielle diffuse et celles qui sont logées dans les corps atrétiques autonomes: chondriome tannophile, lipogénèse, cristallogénèse, système de lacunes et canalicules et d'autres caractères cytologiques de la cellule interstitielle se trouvent indifféremment dans les cellules de la nappe diffuse et dans celles des corps atrétiques autonomes. Cependant, nous n'avons pas pu faire encore un examen assez approfondi de ces deux types de cellules; il est probable que cette similitude ne soit qu'apparente et due à une connaissance superficielle des deux types cellulaires.

<sup>5</sup> Sobotta, Regaud, Popoff, etc.

<sup>6</sup> Pour cette nomenclature voir la note "Sur l'évolution de l'ovaire adulte de la Lapine." *Compt. Rend. de la Soc. de Biol.* No. 30, T. 85.

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## BOOKS RECEIVED

**THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY** (DORLAND), New (11th) Edition, Revised and Enlarged, 1921. First published in 1900. A complete dictionary of terms used in medicine, surgery, dentistry, pharmacy, chemistry, veterinary science, nursing, biology, and kindred branches; with new and elaborate tables. Eleventh edition, revised and enlarged. Edited by W. A. Newman Dorland, M.D. Large octavo of 1229 pages with 338 illustrations, 141 in colors. Containing over 1500 new terms. Philadelphia and London: W. B. Saunders Company, 1921. Flexible Leather, \$7.00 net; thumb index, \$8.00 net.

This dictionary has been used constantly for many years by the research investigators of The Wistar Institute of Anatomy and Biology. For the last five years it has been the Publication Department authority for the preparation of manuscripts printed in the Journals and a valuable guide to correct capitalization (Acrel's ganglion, wolffian ducts, etc.) and the simplified usage of compounds.

The revisions have been so frequent that words coming into special and technical use were soon incorporated. The anatomical names of the Basle Nomina Anatomica terminology have the sign (B. N. A.) after them. The new terminology of the American Society of Bacteriologists is included, and the new bacterial names of this terminology are defined. Other features are: the pronunciation of words, many anatomic tables, chemical symbols and formulas, laboratory and clinical tests, reaction, staining and fixing methods.

The light weight paper, clear print, comparatively small size and round corners, durable and flexible binding, make this a very convenient book for constant reference.

**THE ANATOMY OF THE HUMAN ORBIT AND ACCESSORY ORGANS OF VISION** by S. Ernest Whitnall, Professor of Anatomy, McGill University, Montreal, Canada. Illustrated, 428 pages, index. London: The Oxford Medical Publications, Henry Frowde and Hodder & Stoughton, 1921. \$12.00. The subject-matter of this work originally formed the substance of a series of lectures given to candidates for the Oxford Diploma of Ophthalmology, and is here presented in an amplified and completed form.

**THE BLOOD SUPPLY TO THE HEART** in its anatomical and clinical aspects, by Louis Gress, M.D., C.M., with an Introduction by Horst Oertel, Strathcona Professor of Pathology, McGill University, Montreal. 172 pages, illustrated. New York: Paul B. Hoeber, 1921. \$5.00.

*(Continued on page 240)*

Resumen por el autor, R. A. Boyden

Una vejiga pancreática típica desarrollada a expensas de un  
páncreas accesorio.

En un gato adulto ha hallado el autor un tipo de vejiga pancreática que difiere de todos los casos conocidos hasta el presente por estar relacionada directamente con el duodeno, en vez de las ramas del conducto pancreático. Mientras que en el tipo ordinario se considera derivada de un lóbulo aberrante del páncreas ventral, este ejemplar sin duda alguna se ha desarrollado a expensas de un divertículo accesorio semejante a los descritos en los embriones de cerdo por Lewis y Thyng ('08).

Translation by José F. Nonidez  
Cornell Medical College, New York

## A TYPICAL PANCREATIC BLADDER DEVELOPED FROM AN ACCESSORY PANCREAS

EDWARD A. BOYDEN

*Harvard Medical School, Boston, Massachusetts*

### THREE FIGURES

Although the pancreatic bladder has been recorded at least fourteen times in the domestic cat,<sup>1</sup> it has never been ascribed to any other vertebrate. Even in the cat this anomaly is considered rare, since Johnson ('14) examined nearly four hundred specimens during a period of five or six years without finding an additional case. Miller, who found eight of the fourteen cases reported, suggests in extenuation of his good fortune that there exists a breed of cats in his locality among which pancreatic bladders are of relatively frequent occurrence.

An explanation of this anomaly was early provided through the discovery of two specimens which, although devoid of pancreatic bladders, exhibited accessory lobes of pancreatic tissue in the vicinity of the gall bladder (Miller, '04). In one case this band of pancreas arose from the duodenal division of the main pancreatic duct and extended along the bile duct as far forward as the gall bladder. In the other case there was a small truncated pancreatic mass in the fossa vesicae felleae which communicated with the duodenal division by means of a duct unaccompanied by glandular tissue. These findings were duplicated and extended by Heuer in 1906. Still later Miller ('10) found another specimen combining the two features, namely, a distal pancreatic bladder drained by a duct, the proximal half of which was embedded in a projecting lobe of pancreas. From an analysis of these specimens he concluded that the pancreatic bladder of the adult represents a persistence of the left

<sup>1</sup> See review of literature concluding this paper.

ventral pancreas, which undergoes an aberrant elongation in the direction of the gall bladder. According to this hypothesis, the terminal portion of the lobe becomes dilated, forming the bladder, and the proximal portion atrophies, leaving only a duct. Strong support for this explanation has been offered by Lewis ('11), who described the left lobe of the pancreas, in two pig embryos, as traveling across the ventral mesentery in the direction of the liver, instead of either disappearing, as is normal, or growing around the duodenum to form an infrequent anomaly—the annular pancreas.

The pancreatic bladder discussed in this paper is the second found by the author during the last year. The first of these, in one of twenty-five cats prepared for a class in comparative anatomy, agrees essentially with other cases previously reported. In every instance the aberrant duct arises either directly from the undivided portion of the ductus pancreaticus, or, in close proximity to it, from one of its primary divisions. The second case, found in one of a group of twelve class specimens, is radically different from any other hitherto described. As seen in figure 1, the duct of the pancreatic bladder empties directly into the duodenum some distance below the orifice of the duct of Santorini (*acc.d.*), and it has no relation to the ducts of either the dorsal or ventral pancreas. Throughout its course it is freely patent, and it opens into the duodenum through an orifice which is readily demonstrable, since it is not subdivided by folds such as occur in the cat at the ampulla of the main duct. In the proximal half of its course it is enveloped in a long, slender lobe of pancreatic tissue (fig. 1, *acc.pan.*) which it drains. This lobe is covered by the same peritoneal investment as the head of the pancreas, but is otherwise independent of the main gland from which it projects. As the duct emerges from the lobe, at its duodenal end, it is exposed for a few millimeters, but as soon as it reaches the wall of the intestine it is again enveloped, this time in a layer of fat, which probably represents a part of the original lobe which has here undergone fatty degeneration. In its distal half the duct is free from enveloping tissue. The bladder into which it leads is embedded in the right median lobe of the liver.

It lies to the left of the gall bladder, in which respect it apparently agrees with eight and differs from six of the cases reported. The terminal portions of the two bladders are separated from each other by a lobe of hepatic tissue, but lower down the two bladders are covered by a common fibrous capsule. Both bladders are supplied by branches of the cystic artery, the main trunk of which traverses an isthmus separating the pancreatic bladder into distal and proximal divisions.

In other respects the pancreas is normal; its dorsal and ventral portions are united by a bridge of tissue which completes the ring around the portal vein. According to Heuer, this bridge had formed in about 71 per cent of the cats he examined. The duct of Santorini (fig. 1, *acc.d.*) was present in this specimen and was studied histologically. During its passage through the wall of the duodenum it was found to give off short diverticula—a characteristic which it has in common with the corresponding region of the accessory duct in man. The lobe of tissue (fig. 1, *acc.pan.*) enveloping the duct of the pancreatic bladder was likewise sectioned, and was found to consist of typical pancreatic tissue, but, in the part examined, devoid of islands. It was supplied with branches of the superior and inferior pancreaticoduodenal vessels which penetrated the head of the pancreas to reach the under surface of this lobe.

Interest in this case lies chiefly in the light which it throws upon the origin of pancreatic bladders in general. Two possible modes of development have previously been advocated, the first of which has been discussed at the beginning of this paper. According to this view, a lobe of pancreas having a central duct and abortive alveoli, pushes its way out beneath the bile duct and forms a terminal cyst in close relation with the gall bladder. An alternative origin, suggested by F. T. Lewis, is through the subdivision of the hepatic diverticulum. So regarded, pancreatic bladders are extreme instances of double gall bladder (for which Miller in 1910 said that they might be mistaken), emptying into a subdivided cystic and common bile duct. "The inferior subdivision (pancreatic bladder) has lost its connection with the liver, but has retained its connection with the ventral

pancreas" (Lewis, quoted by Dresbach, '11). In support of this, the frequency of double gall bladders in the cat has been cited. Miller once found such a condition in a brother of a cat with a pancreatic bladder, and in a rather limited number of

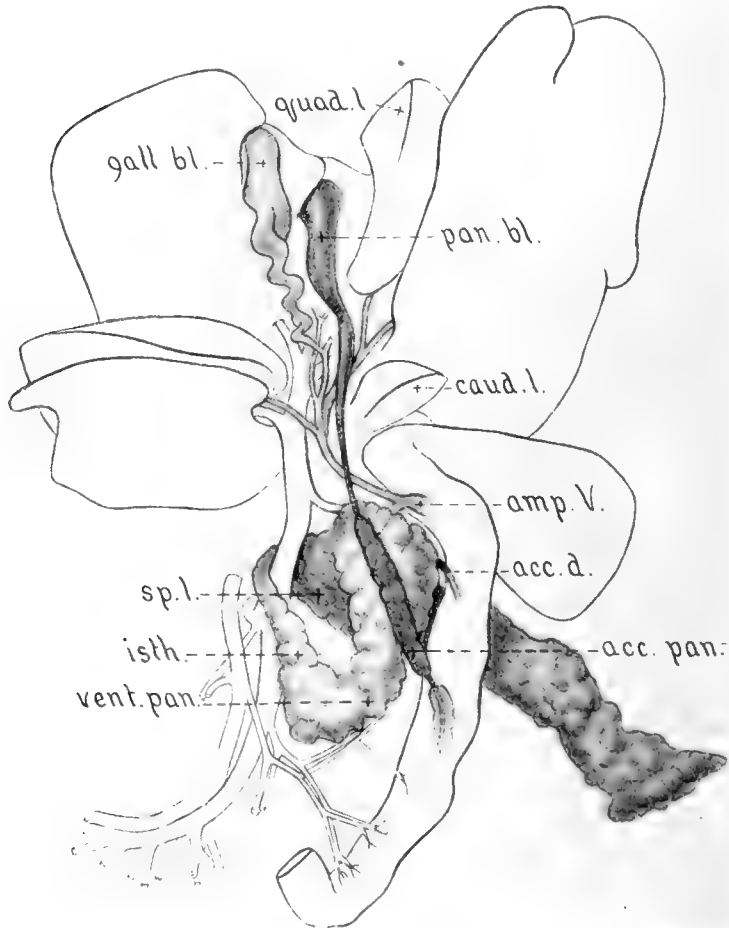


Fig. 1 Pancreatic bladder in a young female cat. *acc.d.*, accessory duct (Santorini); *acc.pan.*, accessory lobe of pancreatic tissue enveloping the duct of the pancreatic bladder; *amp.V.*, ampulla of Vater; *caud.l.*, caudate lobe of liver; *gall bl.*, gall bladder; *isth.*, isthmus connecting dorsal and ventral pancreases; *pan.bl.*, pancreatic bladder; *quad.l.*, quadrate lobe of liver; *sp.l.*, splenic lobe derived from dorsal pancreas; *vent.pan.*, ventral pancreas.



specimens examined a case was recently found by the writer. Notwithstanding certain difficulties which have caused this hypothesis to be discredited by Miller and Lewis, the similarity in structure of gall and pancreatic bladders and their close approximation render it attractive. There are certain cases to

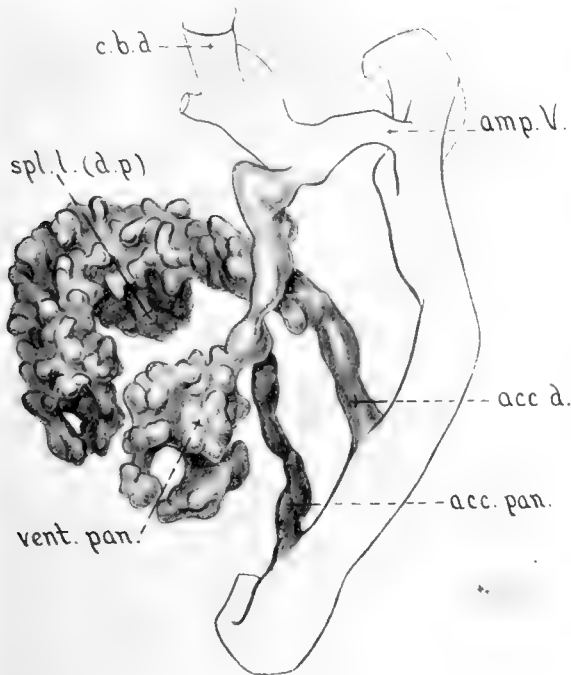


Fig. 2 Accessory pancreas in a pig embryo of 20 mm.  $\times$  55 diam. H. E. C., series 60 (model by F. W. Thyng). *amp.V.*, portion of the bile duct from which the ampulla of Vater is formed; *c.b.d.*, common bile duct. See figure 1 for other abbreviations.

which it may apply, notably the one recorded by Miss Beckwith; and Professor Bremer, at the recent meeting of the American Association of Anatomists, has given new interest to this interpretation by modifying it and correlating it with pancreatic development in the rat. But in other cases, and notably in

the one under discussion, the hypothesis of divided gall bladder can be definitely eliminated.

Since the pancreatic bladder in the present case empties into the intestine below the normal hepatic and pancreatic outgrowths, it must have arisen from an anomalous accessory pancreas well down on the duodenum. Although accessory pancreases in this position have apparently not been recorded in the cat, a short one has been described by F. C. Mann ('20) in the adult dog.<sup>2</sup> As pictured in figure 1 of Doctor Mann's paper, it arises from the duodenum in the region of the duodenojejunal flexure and is directed toward the pylorus, thus corresponding rather closely with the accessory pancreas under discussion, but without terminating in a cystic enlargement. The early development of these anomalous pancreases has been observed in a few embryos, the most suggestive of which, in relation to the present case, is a pig of 20 mm. described by Lewis and Thyng.<sup>3</sup> In this specimen, which was modeled by Doctor Thyng,<sup>4</sup> from whose model a new drawing is here presented as figure 2, there is an accessory diverticulum which not only arises from the same relative position on the duodenum as the aberrant duct in the adult cat, but also travels along the course of the portal vein in the direction of the gall bladder, as far as the dorsal pancreas. It thus gives evidence of being a true accessory pancreas, destined, perhaps, to produce glandular alveoli. In another pig embryo discussed by Lewis and Thyng a similar but shorter diverticulum has become cystic, suggestive of bladder formation, but in that case the expanded terminal portion had become detached and was presumably in process of degeneration. From a study of these pig embryos, it seems probable that the aberrant diverticulum of the cat (fig. 1) represents a corresponding structure which has undergone further development, and having reached the liver has there become expanded into a bladder. Although

<sup>2</sup> Accessory pancreas in the dog. *Anat. Rec.*, vol. 19, p. 265, figs. 1 and 2.

<sup>3</sup> The regular occurrence of intestinal diverticula in embryos of the pig, rabbit and man. *Anat. Rec.*, vol. 7, p. 508.

<sup>4</sup> Models of the pancreas in embryos of the pig, rabbit, cat and man. *Am. Jour. Anat.*, vol. 7, p. 496.

originating as an accessory pancreas, from a primordium clearly distal in position to the normal pancreatic outgrowths, it has nevertheless duplicated the growth and transformation of the left lobe of the ventral pancreas in several of the cases of pancreatic bladder previously reported.

# SUMMARY OF CASES

- 1815 MAYER, A. C. Arch. f. Anat. u. Phys., Bd. 1. Pancreatic bladder to right of gall bladder and smaller; duct terminates in trunk of ductus pancreaticus. This case was given considerable publicity. Cuvier (as noted by Lewis) described it in his *Leçons d'anatomie comparée*, 2<sup>e</sup> édit., vol. 4, part 2, p. 587, Paris, 1835; and Owen, as Doctor Shaner informs us, repeats the description, referring it to Cuvier, but not to Mayer, in his *Anatomy of Vertebrates*, vol. 3, p. 496, London, 1868. No additional case, however, seems to have been recorded during an interval of sixty-four years.
- 1879 GAGE, S. H. Amer. Quart. Mic. Journ., vol. 1, p. 123. Pancreatic bladder to right of gall bladder and larger; duct terminates at the junction of duodenal and gastrosplenic divisions of the ductus pancreaticus.
- 1904 MILLER, W. S. Am. Jour. Anat., vol. 3, p. 269. *Three cases*: pancreatic bladder to left of gall bladder and, in two cases, smaller; duct opens into duodenal division of the ductus pancreaticus, 6 or 7 mm. from its junction with the gastrosplenic division. *Two cases* of pancreatic lobes paralleling the course of the ductus choledochus.
- 1905 MILLER, W. S. Anat. Anz., Bd. 27, S. 119. Pancreatic bladder to left of gall bladder and "of nearly the same size;" duct terminates in gastrosplenic division as above.
- 1906 HEUER, G. C. Johns Hopkins Hospital Bull., vol. 27, p. 106. *Two cases* of pancreatic lobes paralleling the course of the ductus choledochus.
- 1910 MILLER, W. S. Anat. Rec., vol. 4, p. 15. Pancreatic bladder below, to right of gall bladder and (in the figure) of nearly the same size; duct accompanied by pancreatic tissue terminating in duodenal division.
- 1911 DRESBACH, M. Anat. Rec., vol. 5, p. 365. Pancreatic bladder below, to right of gall bladder and smaller; duct joins other divisions in forming common sinus before entering ampulla.
- 1914 JOHNSON, C. E. Anat. Rec., vol. 8, p. 267. Pancreatic bladder to left of gall bladder and smaller; duct terminates at junction of two divisions of ductus pancreaticus.
- 1920 LARSELL, O. Anat. Rec., vol. 18, p. 345. Pancreatic bladder on the left of gall bladder and slightly larger; its main duct empties into the duodenal division. *Three additional cases*, obtained by Professor Miller, are here briefly described. (Two of these had been recorded, without description, by Dresbach, '11.) One was similar in all respects to Miller's second case in 1904 (pancreatic bladder on the left and smaller, emptying into duodenal division of pancreatic duct); the second case "corresponded with the one reported in 1905;" the third was similar to Miller's case of 1910.

1920 BECKWITH, CORA J. *Anat. Rec.*, vol. 18, p. 363. Pancreatic bladder to right of gall bladder and smaller; macroscopically, no trace of pancreatic tissue in connection with the pancreatic bladder or its duct; this duct opens directly into the ductus pancreaticus only 5 mm. from the place where the latter enters the duodenum close beside the ductus choledochus; differs from all previous cases by having an anastomosis between the duct of the pancreatic bladder and the cystic duct; since the cystic duct is occluded beyond the anastomosis, which is a large one, the pancreatic bladder was apparently functioning as a gall bladder. (For the measurement (5 mm.) and for confirming the correctness of this résumé by comparison with the specimen, still preserved in the laboratory of Vassar College, the writer is much indebted to Professor Beckwith.)

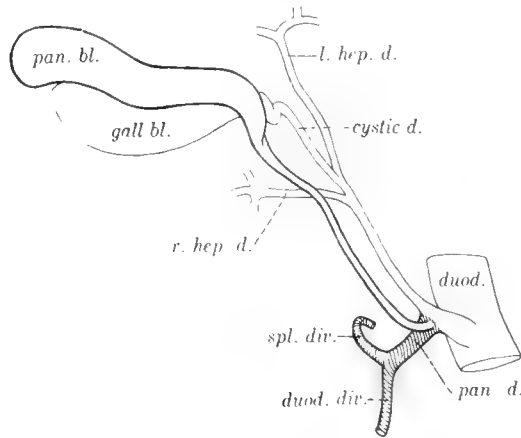


Fig. 3 Pancreatic bladder from a female cat. *duod.*, duodenum; *pan.bl.*, pancreatic bladder; *pan.d.*, pancreatic duct with splenic and duodenal divisions, *spl.div.* and *duod.div.*; *r.hep.d.*, *l.hep.d.*, right and left hepatic ducts; *cystic d.*, cystic duct; *gall bl.*, gall bladder.

The case reported by Miss Beckwith apparently belongs in the same class with the human double gall bladder recorded by Cruveilhier (*Bull. de la soc. anat. de Paris*, 1860, pp. 66, 67). The gall bladder which he described seemed to present merely a bifid fundus, but upon dissection it was found to be double as far as its neck. From the single neck two ducts were given off, one of which emptied into the hepatic duct. The termination of the other, very unfortunately, remains unknown, since it had been cut when the liver was removed at autopsy.

Even though the description is incomplete, it indicates that the specimen is essentially like Miss Beckwith's and that both should perhaps be removed from the group of pancreatic bladders. But if so, should not the following case, which is similar except that it lacks the anastomosis, be placed with them? It appears that the very different conditions of double gall bladder and true pancreatic bladder may be bridged in some instances by specimens which cannot be classified, since as yet a criterion for distinguishing them is not available.

1921 BOYDEN, EDWARD A. (the two cases reported in this paper):

Case 1. Pancreatic bladder to left of gall bladder and larger; its neck crosses that of the gall bladder, ventrally, to gain right side of bile duct; pancreatic bladder gray, empty, with walls thicker than those of the gall bladder; the walls of the two bladders fused throughout entire length and covered by a common fibrous capsule; cavities and ducts of two bladders not in communication with each other; duct of pancreatic bladder terminates in main stem of ductus pancreaticus, just before latter joins the common bile duct (fig. 3).

Case 2. Pancreatic bladder derived from accessory duodenal pancreas (for description, see text of this paper and fig. 1).

#### TOTAL NUMBER OF 'PANCREATIC BLADDERS' REPORTED

- 15 cases, derived either from an aberrant left lobe of the ventral pancreas or possibly from a subdivided gall bladder.
- 4 cases of an accessory lobe of pancreatic tissue paralleling the course of the ductus choledochus (which might be added to the above 15 as incomplete cases of pancreatic bladders).
- 1 case of pancreatic bladder derived unquestionably from an accessory duodenal pancreas.

Resumen por el autor, Shirley P. Miller.

Efectos de varios tipos de inanición sobre las mitocondrias del epitelio gastro-intestinal y pancreático de la rata albina.

La deficiencia en vitaminas y la inanición aguda pueden producir cambios en las mitocondrias de las células epiteliales gastro-intestinales y células glandulares del páncreas. La axfisia no produce aparentemente cambios tan marcados. La intensidad de la lesión sufrida por la célula debe ser muy grande para producir tales cambios. Cowdry ('20) ha llegado a las mismas conclusiones en sus experimentos sobre las raíces de las plantas.

Los cambios observados pueden consistir en: 1 (Una transformación de las mitocondrias bacilares en esféricas; 2) Una aparente reducción en el número de mitocondrias; 3) En la desaparición total de las mitocondrias de las células. A causa de la dificultad para obtener uniformidad en la técnica y las variaciones extremas observadas en las reacciones tintóreas, aún en las células normales, es evidente que debe observarse gran cuidado al derivar conclusiones con referencia a los efectos de los cambios del medio ambiente sobre las mitocondrias.

Translation by José F. Nonidez  
Cornell Medical College, New York

## EFFECTS OF VARIOUS TYPES OF INANITION UPON THE MITOCHONDRIA IN THE GASTROINTESTINAL EPITHELIUM AND IN THE PANCREAS OF THE ALBINO RAT

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Although there is an extensive literature upon mitochondria, the most of this is of a purely morphological nature. But few investigations have been made to determine their modification in number, size, or shape under experimental or pathological conditions. The observations of Lewis' ('15) would indicate that mitochondria are very labile and variable in their morphology. Changes in mitochondria have been described by Homans ('15) in pancreatic islet cells during diabetes; by Scott ('16) in the pancreatic cells of white mice in phosphorus poisoning; by Goetsch ('16) in thyroid cells during goiter, and by Cowdry ('20) in plant rootlets subjected to various harmful conditions. McCann ('18) observed persistence of mitochondria in the nerve cells of monkeys dying of experimental poliomyelitis. Rasmussen ('19) was unable to find any appreciable decrease in the mitochondria in the nerve cells of hibernating woodchucks. Russo ('12) found an increase in the amount of mitochondria in the oöcytes of rabbits during lecithin feeding. Apparently the only published observations on the effects of inanition upon the mitochondria are the results briefly stated by Schun Ichi Ono ('20) at a meeting of the Anatomical and Anthropological Association of China. He demonstrated histological preparations showing the effect of starvation upon the mitochondria in the somatic cells of *Ascaris*, causing them to become granular. He also observed altered size and shape of mitochondria in the tissues of starved rodents. Since further investigation of this question seemed desirable, the present study was undertaken in

order to determine the changes in the mitochondria of the gastro-intestinal epithelium and of the pancreas in the albino rat during various types of inanition. During the progress of this study, it seemed wise to have for comparison some material which had been submitted to other experimental conditions than types of starvation. Therefore, a number of rats were asphyxiated by suffocation.

#### MATERIAL AND METHODS

The thirty-four albino rats used in these experiments were all males except three. The age of the animals ranged from new-born to adult. Three animals were new-born, weighing 4 grams each. Two were mature adults with a weight of about 275 grams. The remainder were young adults weighing from 140 to 160 grams. All animals were weighed at the beginning and end of the experiments. The lengths of the body and the tail were taken at the time of death. The age of all animals was known. As far as possible, test animals and controls were from the same litter, and were killed at the same time. The animals subjected to starvation were killed in the advanced stages of inanition, most of them when the animal was near death.

The animals submitted to asphyxiation were placed on a glass plate and covered with a bell jar of about 1000 cc. capacity. The chamber was made air tight by means of vaselin spread around the juncture of plate and bell jar. Six to seven hours elapsed before the rats were asphyxiated.

The thirty-four rats were divided into the following groups: nine were given no food, but allowed water; one was given neither food nor water; one was a new-born and not allowed to nurse.

Through the kindness of Prof. J. F. McClendon, four rats were obtained which had been fed on diets deficient in water-soluble A vitamine in various degrees. I am likewise indebted to Prof. R. Adams Dutcher for two rats, one fed upon an exclusive diet of gelatin for thirty days and the other for the same length of time on an exclusive diet of zein. Seventeen normal rats were used as controls.



The tissues of both test rats and control animals were fixed in the same manner and simultaneously dehydrated and imbedded in paraffin.

Bensley's acetic-chromic-osmic and Regaud's formalin-bichromate fixers were used. Sections were cut 2 and  $3\mu$  thick, and mounted in a series of ten or twelve to a slide. In some cases test and control tissues were mounted on the same slide—a method which gave the same staining technique for each.

The sections were stained by the acid-fuchsin-methyl green method of Bensley.

#### OBSERVATIONS

At the outset it may be well to emphasize the great variation found in the form and number of mitochondria in both the test and control tissues, due in part to the difficulty in securing uniformity of technique. Not all the cells of the epithelium of the test animals exhibit the modifications which are described. There are areas in the epithelium in which the cells show evidence of necrosis as a result of injury due to experimental conditions. In such circumscribed regions mitochondrial changes in form and number were apparent, especially in the deeper portions of the mucosa.

*The normal stomach.* In the gastric glands the mitochondria of the chief cells are short, straight rods. In most of the cells one may also find spherical mitochondria, but only a few to a cell. The spherical forms present the appearance of having been separated from the rod-like forms. Occasionally there is a linear arrangement of such spheres, as if a whole rod had become segmented. The rods vary from 2 to  $5\mu$  in length and the spheres from five-tenths to eight-tenths of a micron in diameter. The mitochondria are in most cells uniformly distributed throughout the cytoplasm. In exceptional cases there is a peripheral condensation, or grouping of the mitochondria, chiefly at the base of the cell.

Because of the difficulty in distinguishing between the acidophile secretory granules and the mitochondria of the parietal cells, no special study was made of these cells. Satisfactory

results in case of these cells could be obtained only by the use of the intra vitam stains. The cells of the foveolae and the surface epithelium have distinctly more mitochondria in them than do those of the glands. The mitochondria are uniformly distributed throughout these cells, and the size of the mitochondria is very nearly the same as of those in the chief cells.

*The stomach of test rats.* A study was made of the stomach in four rats fed for periods from 89 to 105 days on a diet variably deficient in water-soluble A vitamine. The chief cells of the gastric glands, in areas evidencing injury, exhibit changes in the mitochondria. This is especially true in case the diet is markedly deficient in the vitamine. The rod-like forms of mitochondria are entirely lacking, although a few spherical mitochondria occur. The cytoplasm of such cells exhibits a fine vacuolization at the periphery towards the lumen of the gland. Occasionally one finds this vacuolization throughout the cell. In the surface epithelium and the gastric foveolae the mitochondria show no well-marked or constant changes.

In the gastric glands of the two rats which had been fed on a diet of gelatin or zein, no mitochondria were found in the chief cells of the rather extensive necrotic areas.

Similarly in the rats subjected to acute inanition and in the last stages of starvation, no mitochondria occur in the chief cells of degenerated areas. Even in areas not exhibiting marked degeneration there are few if any mitochondria to be noted.

The cells of the foveolae and the surface epithelium, in all the experimental rats mentioned above, do not show as marked changes in the mitochondria. In those of the acute starvation series the rods are replaced to some degree by spheres. In but very few cases was it possible to find marked changes in the mitochondria of the cells of the foveolae and surface epithelium.

Observations upon the stomachs of asphyxiated rats show similar changes in the mitochondria of the chief cells, especially in areas evidencing injury. There are no rods, but spheres are present and apparently in reduced number. The cytoplasm of such cells is more or less vacuolated, resembling this condition in the cells of the other experimental animals. The cells of the

foveolae and the surface epithelium show no changes in the mitochondria.

*The normal duodenum and pancreas.* In the normal rats the mitochondria in the cells of the glands of Lieberkühn of the duodenum resemble those of the chief cells of the stomach. They are somewhat shorter, however, measuring from 2 to 4 $\mu$  in length. Their distribution in the cells is likewise similar to that of the chief cells of the stomach. The epithelial cells of the villi resemble those of the foveolae and surface cells of the stomach epithelium, in so far as the mitochondria are concerned.

The mitochondria of the pancreas cells appear as long, straight rods. Their length is about twice that of those in the chief cells of the stomach. They are uniformly distributed throughout the protoplasm in the vicinity of the nucleus. The protoplasm of the cells nearest the lumen of the pancreatic alveolus contains secretory granules more frequently than it does mitochondria.

In the rats fed upon a vitamine-deficient diet the gland cells of the duodenum show spherical more frequently than rod-like mitochondria. Very often both spheres and rods occur in the same cells, but the rods are always fewer in number.

In acute inanition the mitochondria are usually absent in the cells of the glands of Lieberkühn and in the villus epithelium they are apparently decreased in number.

In the asphyxiated material there is no appreciable change in the mitochondria in the cells of either the glands or the surface epithelium of the villi.

The pancreas of the experimental animals does not exhibit abnormal areas in which the cells show evidences of injury such as occur in the stomach and duodenum. In vitamine deficiency the mitochondria are present in the gland cells, but the number of rods is decreased, most of the mitochondria being spherical in form. In the pancreas of animals suffering from acute inanition no rods occur. All of the mitochondria appear spherical in shape. Asphyxiation does not appear to modify the mitochondria in shape or number.

Because of the minuteness of the mitochondria in the pancreatic islet cells, no special study of them was attempted in these cells.

## CONCLUSIONS

Vitamine deficiency and acute starvation may produce changes in the mitochondria in the gastro-intestinal epithelial cells and in the gland cells of the pancreas. Asphyxiation apparently does not produce such marked changes.

The amount of injury to the cell must be rather severe in order to bring about such changes. Cowdry ('20) reached the same conclusion from experiments upon plant rootlets.

The changes observed may involve: 1) a transformation of mitochondria from rod-like to spherical forms; 2) an apparent reduction in number of the mitochondria; 3) or even the total disappearance of mitochondria from the cells.

Because of the difficulty in obtaining uniformity of technique and the extreme variations observed in the staining reactions even in the normal cells, it is evident that great caution should be observed in drawing conclusions as to the effect of environmental changes upon mitochondria.

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Resumen por los autores, H. Cummins y J. Sicomio

Un caso de hiperdautilismo; duplicación bilateral del pulgar y primer metatarsiano de un negro adulto.

En este trabajo se describen los dos piés, ambos con seis dedos. Los dos dedos más mediales de cada pié son los pulgares. Las líneas de fricción son semejantes en los dos pulgares de cada pié. Las líneas en la planta no están modificadas. Las falanges de los pulgares y el primer metatarsiano están por completo duplicados. En los tendones del extensor y flexor largos de los pulgares existe una bifurcación, para su inserción en los dos pulgares, y en el pié izquierdo solamente, el vientre más medial del extensor corto de los dedos y el adductor están también bifurcados. En el pié derecho el abductor, adductor, y el flexor corto de los dedos se insertan solamente sobre el pulgar medial; en el izquierdo el abductor y el vientre medio del flexor corto de los dedos se insertan solamente sobre el pulgar medial, junto con una pequeña contribución del adductor, mientras que este último casi entero y todo el vientre lateral del flexor corto de los dedos están relacionados con el pulgar lateral. El curso de los vasos y nervios no presentan cambio que pudieran relacionarse germinalmente con el hiperdautilismo. Se considera a la anomalía descrita como el resultado de una variación germinal solamente en relación con la iniciación de su desarrollo. Como consecuencia de la inestabilidad germinal tuvo lugar, suponen los autores, una reproducción de los determinantes de los huesos del pulgar y de la epidermis durante la vida embrionaria temprana. Con la ulterior diferenciación de los músculos, vasos y nervios estas estructuras se adaptaron, imperfectamente en el caso de los primeros, al doble movimiento del pulgar.

Translation by José F. Nonidez  
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## A CASE OF HYPERDACTYLISM: BILATERAL DUPLICATION OF THE HALLUX AND FIRST METATARSAL IN AN ADULT NEGRO

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### NINE FIGURES

Among the cadavers received in the anatomical laboratories of Tulane University in 1921 there was one hyperdactylous subject. The subject was a male negro, aged about fifty years, who died of chronic interstitial nephritis and chronic pleurisy. The body exhibited no external abnormalities other than the occurrence of six digits on each foot. Skiagrams identified the extra toes as halluces, and moreover demonstrated complete duplication of the first metatarsal in either foot. In spite of the large number of hyperdactylous cases reported, the opportunity for dissection of such material is relatively rare, especially in the adult. For this reason, and because of the unusual completeness of duplication in the case described, the results of its study were thought to be worthy of record. Information relating to the subject in life is not available. Therefore it is impossible to trace the inheritance of hyperdactylism in his family or to ascertain the degree of usefulness or inconvenience occasioned by the added toes.

Effective embalming of the body had been accomplished before the feet were removed. With the aim of handling them more readily in subsequent study, the feet were amputated at a level of about 5 cm. above the ankles. Although the legs were retained, to be dissected in the event that atypical muscles or other structures would require tracing upward, the occasion for dissection at higher levels did not arise. Obviously, actual prints would have been ideal material for the observation of epidermal patterns. But the hardening consequent to embalming, which

rendered every surface irregularity incompressible, and desquamation of the superficial epidermis proved to make successful prints impossible. Casting of the soles in wax was likewise unsuccessful. It was finally necessary to resort to direct study of the plantar skin and the use of drawings as records for publication. These drawings, reproduced as figures 3 and 4, are based upon careful observation of the patterns under a hand lens. Lines of interpretation were followed with ink directly upon the skin. In drawing, the interpretation lines were first inserted, their locations within the sole outline being determined by measurement; then the courses of cristae within individual areas were added in a diagrammatic manner. Wilder's paper of 1916 was used as a guide in the study of these patterns. The method of dissection requires no comment. Full notes were made as the dissection progressed, and at the same time figures 7, 8, and 9 were made in their present form, drawn by one of us and checked by the other. In studying skeletal parts use was made of the skiagrams as well as the actual bones. While other texts on descriptive anatomy were consulted, Morris' Human Anatomy was used as a guide. Lymphatics, deep veins, and ligaments were not dissected, but other structures were dissected in entirety in both feet. In many cases no mention has been made of structures which are typical, but in no case has note of a variation been omitted.

The terms *medial hallux* and *lateral hallux* are here used because each is a complete digit and of hallucal identity. In the same manner, the terms *medial first metatarsal* and *lateral first metatarsal* are applied to the bones which represent a doubled first metatarsal. *Interhallucal* is, in the restricted sense which its meaning implies, comparable to interdigital. Enumeration of the typical digits, of the metatarsals associated with them, of the interosseous spaces—in short, of all structures which are numbered in ordinary description of the normal foot—follows the terminology of descriptive anatomy.

Prof. H. H. Wilder has examined the material on epidermal patterns, including the plantar skin from both feet and our drawings and descriptions. The writers are indebted to him for



this kindness and to Dr. A. Henriques, of New Orleans, for the skiagrams.

#### EXTERNAL FEATURES

In their superficial appearance both medial and lateral halluces conform to the nature of fully developed digits and, further, they bear the characteristics of halluces. The medial hallux is a hallux without question, because of its large size, medial position, single phalangeal joint, and broad heavy nail. The lateral hallux is no less a great toe; in spite of its smaller size and aberrant position, it possesses the shape, single phalangeal joint, and broad nail of a hallux. In either foot the lateral hallux is in correct linear relation to the long axis of the foot. On the right foot the medial hallux is medially rotated, although its long axis is in line with the other digits. The left medial hallux is in such pronounced abduction as to project almost vertical (in a horizontal plane) to the long axis of the foot. These differences in position are to be explained below on the basis of variations in distribution of muscle insertions to the right and left medial halluces. Pronounced webs occur in all the interdigital areas of either foot and in the interhallucal area of the right foot, but not in that of the left. Owing to its supernumerary metatarsal and digit, the distal portion of each foot is wider than normal. On the medial margin, of the right foot only, there is a large prominence produced by the head of the medial first metatarsal. Owing to the hardening after embalming, any accurate determination of the contact area in life is impossible. Contact areas, judging from the hardened feet, must have been limited, restricted in size by the unusual height of the arch. Prints of the contact areas are reproduced in figures 1 and 2, which also show the general contour of the feet and positions of the digits.

Epidermal patterns are represented in figures 3 and 4. Notwithstanding slight differences in details of configuration, the hallucal patterns are alike in the two feet. The pattern consists of a single triradius and an open field, corresponding to Wilder's type BC. The first interdigital area in either foot is a loop. In the right foot the narrowed proximal portion of the loop curves

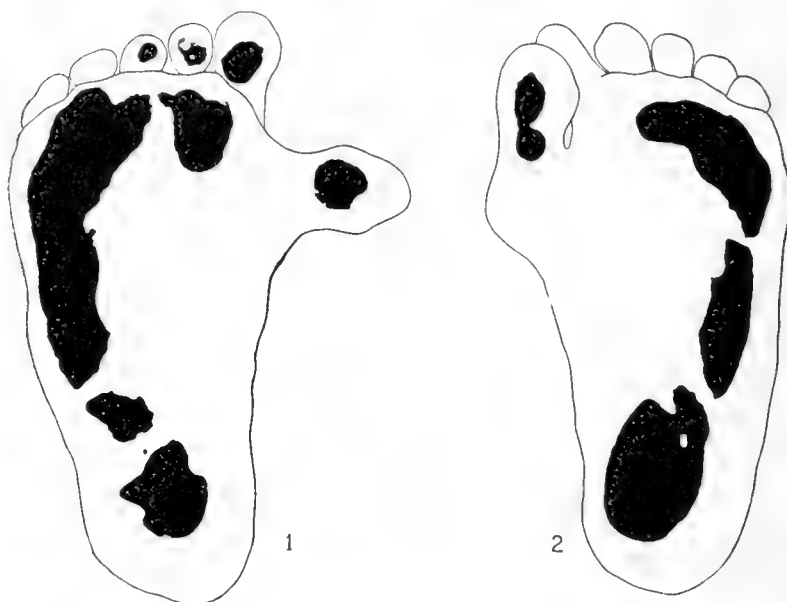


Fig. 1 Contact area of the left foot; an impression made by firmly pressing the completely inked sole of the hardened foot upon a rigid plane surface.

Fig. 2 Contact area of the right foot; made as above.

*Measurements (in millimeters)*

	RIGHT	LEFT
Greatest length of foot, measured from posterior extremity of heel to the furrow at bases of digits.....	211	209
Width of sole, in region of heads of metatarsals.....	115	111
Width of sole, in region of ankle.....	71	78
Greatest diameter of medial hallux.....	31	31
Diameter of medial hallux at its base.....	22	33
Length of medial hallux.....	44	45
Greatest diameter of lateral hallux.....	18	23
Length of lateral hallux.....	31	31
Maximum elevation of roof of arch, above level of contact area.....	26	28
Elevation of apex of lateral malleolus, above level of contact area.....	94	84
Elevation of apex of medial malleolus, above level of contact area.....	92	82

around the lateral border of the second interdigital area, and its ridges then become continuous with the transversely placed ridges in the depressed region of sole area at the bases of the toes. In the left foot the loop becomes confluent with the third interdigital area. In both feet the third interdigital area is an open field; in the right foot it opens at the distal margin of the

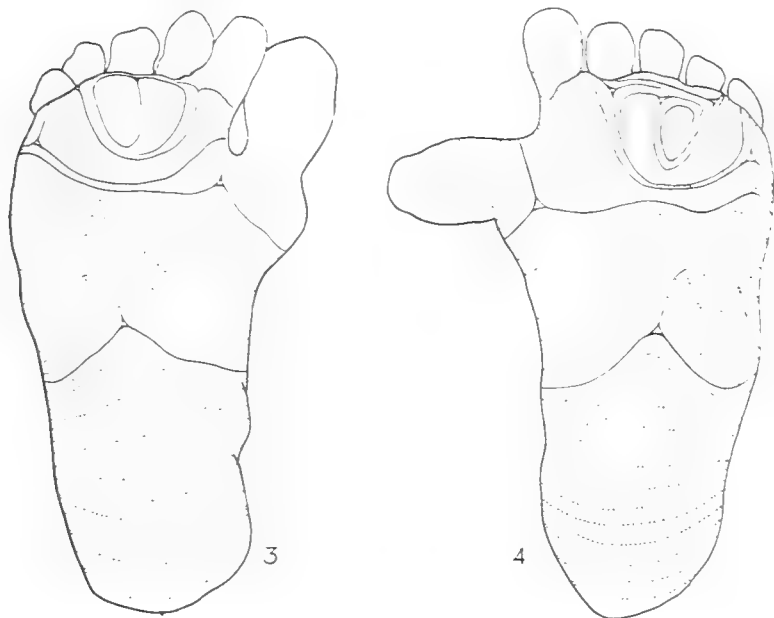


Fig. 3 Epidermal patterns of the sole, right foot; triradii and interpretation lines unbroken; courses of friction ridges shown in dotted lines.

Fig. 4 Epidermal patterns of the left foot, including digital patterns of the halluces; drawn as in the right foot.

sole area lateral to the hallucal pattern, and in the left flows into the loop of the first interdigital area. An extensive hypothenar pattern occurs on each foot, the figure being a large loop and triradius, with the opening of the loop lateral. This pattern is within the tread area. A hypothenar loop occurs in a little over half of all known sole prints. When present it usually opens medially, and often is not within the tread area. The pattern in this case thus is an unusual one with reference to its

lateral opening and its position well down on the sole. A rudimentary thenar pattern is present on each foot, indicated by the divergence of friction ridges as they course toward the medial margin of the sole. No remnant of a calcar pattern is visible. Friction ridges on the medial and lateral halluces of the one foot are counterparts of each other, and they are alike on the two feet. They are arranged in straight transverse lines at the bases of the halluces; distally they become arciform, with the convexity distal. No indications of digital triradii are to be found. Formulation of the patterns follows:

Left foot: BC. L. W. O. H.  
Right foot: BC. L. + III. W. O. H.

#### BONES

In their morphology and number the phalanges of the medial and lateral halluces are typical in both feet. And each of the two first metatarsals of each foot is in size and morphology like the normal single first metatarsal (skiagrams, figs. 5 and 6). Distal phalanges of the right and left medial halluces are similar in size and shape, as are the distal phalanges of the lateral halluces. The proximal phalanx of the left medial hallux is larger than that of the right. On the left foot, the proximal phalanges of the two halluces, at their point of articulation with the corresponding metatarsals, diverge from each other, while on the right foot they converge. The proximal phalanx of the right medial hallux is medially rotated upon its long axis. In the left foot, the facet on the medial first metatarsal for articulation with the proximal phalanx of the medial hallux is shifted proximomedially, and in the right foot the corresponding facet is situated proximolaterally. The mutual contact surfaces of the two first metatarsals are more extensive in the left foot than in the right. In the left foot, articular surfaces and joint capsule are lacking in the region of contact, the metatarsals being held together by dense connective tissue in amount great enough to allow very little movement, if any. In the right foot, articular surfaces and joint capsule occur at this junction. Proximally, the medial first metatarsal of each foot articulates with the first

cuneiform, but the articular facet of the right one faces farther proximolaterally than does the similar facet of the left. The lateral first metatarsal of the left foot articulates proximally with the first cuneiform and with an accessory bone. This accessory bone is a triangular pyramid in shape, its base facing dorsally. It is wedged between the distal extremities of the first and second cuneiforms, articulating with them as well as with



Fig. 5 Skiagram of the left foot; rayed from the dorsal aspect.

Fig. 6 Skiagram of the right foot; rayed from the dorsal aspect.

the lateral first metatarsal. The lateral first metatarsal of the right foot is extended proximally as a wedge-shaped process fitting between the distal extremities of the first and second cuneiforms. If the process were transversely separated from the body of the metatarsal, it would have the same size, shape, and articulations as the accessory bone of the left foot. From their point of articulation with the tarsus, the medial and lateral first metatarsals of the right foot diverge, and this divergence is rendered apparently greater by the constriction of their shafts. Although there is

some divergence of the same bones in the left foot, the angle is not so great and is apparently lessened through the more slight constriction of their shafts. In either foot there are sesamoids related to the heads of both first metatarsals.

#### MUSCLES

*M. abductor hallucis* in both feet is restricted in insertion to the medial hallux, with no indication of even a diffused or aponeurotic insertion upon the lateral hallux. The area of insertion, at the base of the proximal phalanx, is typical. The origin of the abductor is typical, except that a larger than usual proportion of its fibers originate from the fibrous arch on the deep surface of the muscle; the arch itself is of uncommonly great proximodistal extent.

*M. flexor hallucis brevis* in either foot is divided, as usual, into medial and lateral bellies. LEFT: The lateral belly is much the larger of the two. It arises in common with the adductor from the first, second, and third cuneiforms, from the latero-plantar aspect of the base of the lateral first metatarsal, and from the peroneus longus tendon, but not from the plantar calcaneocuboidal ligament. Insertion of the lateral belly is confined to the lateral hallux, at the base of its proximal phalanx. The medial belly has a dual origin, from the first cuneiform and from the fibrous arch on the deep surface of the abductor. It is readily separable into two fasciculi, medial and lateral. The origin of the lateral fasciculus is from the first cuneiform; its tendon of insertion accompanies and is fused with those of the medial fasciculus and abductor, ultimately inserting upon the medial aspect of the proximal phalanx of the medial hallux. The medial fasciculus arises only to a limited extent from the first cuneiform, most of its fibers originating in common with that portion of the abductor which comes from the fibrous arch on its deep surface. It inserts in common with the lateral fasciculus and abductor, wholly upon the medial hallux. RIGHT: The lateral belly is intimately fused with the oblique head of the adductor, and inseparable from it. Insertion, like the adductor of this foot, is confined to the medial hallux. The medial belly

is fused throughout its length with the abductor, is not separable into two fasciculi, and inserts wholly upon the medial hallux.

*M. adductor hallucis* in each foot is separated into the usual transverse and oblique heads, both of typical origin. LEFT: Tendons of insertion from the two heads are fused together and, with the tendon of the lateral belly of the flexor hallucis brevis, are inserted upon the base of the proximal phalanx of the lateral hallux. In addition, the oblique head gives rise to a small tendon which, between the bases of the proximal phalanges of the two halluces, bifurcates into a smaller medial division and a larger lateral one. The medial division inserts directly upon the plantar aspect of the proximal phalanx of the medial hallux at its base. The lateral division is continued into the longitudinal tendinous band in the tendon sheath of the flexor hallucis longus, described under that muscle. RIGHT: The muscle is much larger than in the left foot, especially its transverse head. Insertion is confined to the medial hallux.

*M. flexor hallucis longus*. LEFT: The tendon of insertion is bifurcated and inserts upon the bases of the distal phalanges of both halluces, as shown in figure 9. Its synovial sheath is likewise divided as it accompanies the two divisions beyond the common tendon. Proximal to the point of bifurcation, on its plantar surface only, the common tendon is superficially divided into medial and lateral halves by a slight longitudinal furrow, visible almost to the point of fusion of this tendon with that of the flexor digitorum longus. When the common tendon is forcibly split along the line of this furrow, small bundles of tendon fibers of the two components are found to interlace, but in spite of their presence the separation of the common tendon clearly follows the superficial furrow. The relation of the common tendon to the flexor digitorum longus is described under the latter muscle. In the dorsal aspect of the sheath which encloses the tendon to the lateral hallux there is a condensed longitudinal band of tendinous fibers. Proximally, most of these fibers are attached to the base of the proximal phalanx of the lateral hallux, but a few radiate medially to reach a similar attachment upon the medial hallux. Distally, this band is fused to the base

of the distal phalanx of the lateral hallux. A contribution to the band from the oblique head of the adductor has been noted above. RIGHT: The tendon is slightly fused with that of the flexor digitorum longus, at the point where it is crossed diagonally by the latter. Distal to the level of fusion the flexor hallucis longus tendon divides into three bundles, of which the one most medial is the largest. This medial bundle extends distally as far as the middle of the two first metatarsals, where it bifurcates into medial and lateral tendons; these insert upon the medial and lateral halluces, respectively. The tendon to the medial hallux is larger than the lateral one. The intermediate one of the three bundles, along with the above-mentioned lateral tendon of the medial bundle and with a slip from the flexor digitorum brevis, inserts upon the lateral hallux. The lateral bundle simulates a tendon of the flexor digitorum longus; it inserts upon the second digit and provides origin for the first and second lumbricals.

*M. extensor hallucis longus.* LEFT: The tendons of this muscle are represented in figure 7. Its tendon of insertion is bifurcated, and an aponeurosis is stretched between the two divisions. For the most part, the fibers of the common tendon are continued distally to insert upon the phalanges of the lateral hallux, although some fibers are contributed to the aponeurosis. The aponeurosis is attached not only to the two divisions of the extensor tendon, but also to the opposed surfaces of the proximal phalanges of both halluces and to the heads of both first metatarsals. The division to the medial hallux is aponeurotic near the common tendon, with indications of four condensed bundles of fibers which become convergent as they extend distally; insertion is at the base of the distal phalanx. The common tendon sends a long thin slip to that tendon of the extensor digitorum longus which inserts upon the second digit. RIGHT: In this foot the common tendon bifurcates, but the divisions are much more distinctly separate than in the left. Distally there is no aponeurosis stretched between the two divisions, and each division is a clearly separate tendon with normal insertions upon the phalanges of either hallux. Proximally, in the angle between the



two divisions, there is a slight aponeurosis which is not attached to the adjacent bones as in the left foot.

*M. extensor digitorum brevis*. LEFT: The most medial belly, sometimes called extensor hallucis brevis, possesses a tendon which bifurcates into medial and lateral divisions, with an aponeurosis between them. The medial division enters the aponeurosis of the extensor hallucis longus, and as a distinct fasciculus

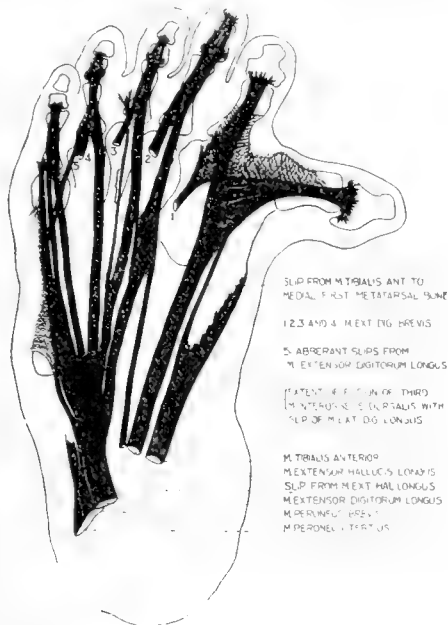


Fig. 7 Tendons of the dorsum, left foot.

is continued distally to insert upon the medial hallux at the base of its proximal phalanx. The lateral division inserts upon the head of the lateral first metatarsal and base of the proximal phalanx of the lateral hallux. The aponeurosis is continuous with that of the extensor hallucis longus, and loses its identity within the common aponeurosis thus formed. RIGHT: Here the muscle belly is typical, and inserts wholly upon the lateral hallux.

*M. flexor digitorum brevis* is normal in the left foot, but in the right possesses five bellies. The four lateral ones apparently represent the typical muscle, while the most medial belly inserts upon the lateral hallux.

*M. flexor digitorum longus.* LEFT: This muscle sends a slip from its tendon to that of the flexor hallucis longus, the level of origin of the slip being slightly proximal to the insertion of the quadratus plantae (superficial portion of the medial head) into the tendon of the flexor digitorum longus. The position and direction of fibers in the slip indicate that they are destined to reach the medial hallux only. There are two distinct strata in the tendon, superficial and deep. The deep stratum is composed of fibers issuing from the tendon of the flexor hallucis longus, at about the same level as the slip to this tendon, and continuing distally as two tendons inserting upon the second and third digits. Tendons to the fourth and fifth digits are from the superficial stratum, the one to the fifth digit being small and rudimentary. RIGHT: Coincident with the replacement of its most medial tendon by a slip from the flexor hallucis longus, this muscle has only three tendons of insertion. The most medial of the three sends a slip into the lateral bundle of the flexor hallucis longus. Also small bundles of fibers pierce directly through the tendon of the flexor hallucis longus, and are continued distally as longitudinal fasciculi to reenter the tendon to the third digit. The most lateral of the three tendons is unusually large; it receives a slip from the flexor digitorum brevis.

*M. quadratus plantae* in either foot is divided into the usual medial and lateral heads. LEFT: The medial head is separated into two strata, superficial and deep. Insertion of the former stratum is upon the lateral margin of the plantar surface of the flexor digitorum longus tendon, superficial portion. The deep stratum inserts upon the lateral margin of the deep portion of the same tendon. The lateral head inserts like the deep stratum of the medial head, but more distal. RIGHT: Insertion of the medial head is upon the tendon of the flexor digitorum longus, on its lateral margin and dorsal aspect just proximal to the trifurcation of the tendon. The lateral head inserts upon the

lateral margin of the lateral bundle of the flexor hallucis longus and upon the three tendons of the flexor digitorum longus.

*M. peroneus longus* in either foot is typical, but that portion which normally inserts upon the first metatarsal is here limited to the medial first metatarsal.

*M. interossei* do not occur between the two first metatarsals of either foot.

#### SUPERFICIAL VEINS; ARTERIES

The courses of superficial veins are alike in the two feet, with no deviations of note other than provision for drainage of both halluces. These vessels of the dorsum of the left foot are shown in figure 8.

*A. dorsalis pedis.* LEFT: The first dorsal metatarsal (a. dorsalis hallucis) arises in line with the navicular, so far proximal as to carry the medial tarsal branch with it. Its primary distribution is normal. A branch to the deep structures in the interosseous space between the two first metatarsals arises from it, in line with the bases of the first metatarsals. It gives no supply to the medial hallux. RIGHT: General relations of a. dorsalis pedis are as in the left foot, but the second dorsal metatarsal does not originate from the dorsal arch. Instead, it comes from the plantar arch, issuing through the proximal extremity of the second interosseous space, to be distributed in the manner of a second dorsal metatarsal.

*A. plantaris lateralis and ramus plantaris profunda.* LEFT: The former vessel is of uncommonly small diameter. From it the following branches originate, in order beginning laterally: 1) A branch which courses distally on the plantar surface, bifurcating, in line with the head of the fifth metatarsal, into medial and lateral terminals. The lateral supplies the lateral aspect of the fifth digit and the adjacent plantar surface and lateral margin of the foot. The medial terminal supplies the opposed surfaces of the fourth and fifth digits. 2) A metatarsal supplying the opposed surfaces of the third and fourth digits. 3) A branch supplying the deep structures adjacent to the distal portions of the first and second interosseous spaces. 4) The

continuation of the arch medial to the last-named branch is an extremely small artery, which, on the lateroplantar aspect of the base of the lateral first metatarsal, joins the deep plantar branch of the dorsalis pedis. From the medial aspect of this junction there is an artery, which, because of its large size and linear relation to the deep plantar, is considered to be a continuation of the deep plantar rather than of the plantar arch. This artery continues medially as far as the line of contact between the two first metatarsals, where it turns distally in the interosseous space between them and is joined by the 'deep branch proper' of the medial plantar. The common trunk distal to the junction, larger in diameter than would be expected of a metatarsal, is distributed to the interhallucal area and opposed surfaces of the two halluces. RIGHT: As in the left foot, the contribution of the lateral plantar artery to the plantar arch is reduced not only with reference to the diameter of the vessel, but also as to the distance which it extends medially and the number of branches originating from it. There are three branches of the lateral plantar; they are, in order beginning laterally: 1) A branch to the lateral aspect of the fifth digit and the adjacent plantar and lateral region of the foot. 2) A branch which perforates the proximal extremity of the first interosseous space and is distributed as a dorsal metatarsal (noted under a. dorsalis pedis). 3) The lateral plantar artery terminates by anastomosing with the lateral trunk of the deep plantar. The deep plantar issues through the proximal end of the first interosseous space, dividing immediately into two large trunks, lateral and medial. The lateral trunk gives rise to two branches, lateral and medial, at the point of junction of this trunk with the lateral plantar. The lateral branch almost at once divides into two plantar metatarsals, coursing, respectively, in the fourth and third interspaces. The medial branch is distributed as a plantar metatarsal to the second interspace. Distally, the most medial two of the three plantar metatarsal branches receive anastomoses from the superficial branch of the medial plantar. The medial trunk crosses the base of the lateral first metatarsal, and then turns distally in the interosseous space between the two first metatar-

sals; at the middle of this space it divides into two branches, plantar and dorsal. The plantar branch anastomoses with the deep branch of the medial plantar; beyond the anastomosis it runs superficial to the insertion of the adductor hallucis, and then anastomoses with the dorsal branch. Two digital branches, distributed to the opposed surfaces of the halluces, arise from the plantar branch. The dorsal branch turns dorsally in the interhallucal area, first giving rise to a branch reaching the base of the medial hallux, then arching toward the plantar surface, where it enters into anastomosis with the plantar branch. In its course toward the plantar surface it gives origin to a digital branch to the medial side of the lateral hallux. It is evident that the anastomosis between the dorsal and plantar branches of the deep plantar results in the formation of an arterial ring which also receives a contribution from the deep branch of the medial plantar. Both heads of the adductor and the lateral belly of the flexor hallucis brevis are encircled by the ring, near their insertions upon the medial hallux.

*A plantaris medialis.* LEFT: From the superficial branch there are two branches of especial interest in that they replace two of the metatarsal arteries typically originating from the plantar arch. These two arteries supply, respectively, the opposed surfaces of the second and third digits and the opposed surfaces of the lateral hallux and second digit. The deep branch, in line with the middle of the medial first metatarsal, divides into a deep branch proper and a medial digital branch. The latter supplies the medial and plantar aspects of the medial hallux. The deep branch proper continues distally, ultimately joining the deep plantar artery. RIGHT: From the superficial branch there are three small branches anastomosing with the most medial three plantar metatarsals. The deep branch supplies a digital branch to the medial hallux.

#### NERVES (figs. 8 and 9)

The *sural* in either foot is a little larger in diameter than usual. It descends on the lateral border of the tendon Achilles, posterior to the lateral malleolus, and before turning distally

it gives a large branch which fans out on the lateral surface of the heel; this branch takes the place of the usual lateral calcaneal branches. Distal to this branch the sural is continued along the lateral border of the foot as the lateral dorsal cutaneous nerve, supplying many small branches to the region through which it courses. Ultimately the nerve divides into two dorsal digital branches, one for the lateral surface of the fifth digit and one for its dorsum.

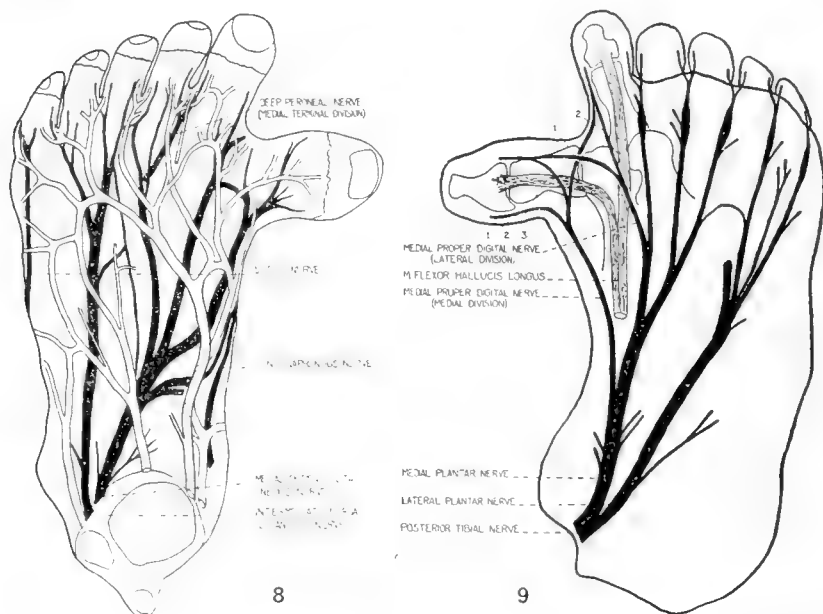


Fig. 8 Superficial veins and nerves of the dorsum, left foot; veins in open lines and nerves solid.

Fig. 9 Plantar nerves and tendon of the flexor hallucis longus, left foot.

The *intermediate dorsal cutaneous* is alike in both feet, with one exception. The nerve divides distally into three dorsal digital branches: the first, from lateral to medial, innervates the adjacent sides of the fourth and fifth digits; the second, the adjacent sides of the third and fourth digits, and the third, the medial side of the third digit. Slightly proximal to the point of origin of these branches, the common trunk gives rise to a prominent

branch which anastomoses with a branch of the medial dorsal cutaneous, and this combined nerve anastomoses distally with the third dorsal digital branch of the intermediate dorsal cutaneous. These anastomoses are lacking in the right foot.

The *medial dorsal cutaneous* divides into five branches in the left foot, only four in the right, the branch lacking in the right foot being the one entering into the anastomosis mentioned above. Beginning laterally, the first branch supplies the lateral surface of the second digit; the second and third branches anastomose distally and innervate the medial surface of the lateral hallux, the interhallucal area and the lateral, dorsal and medial surfaces of the medial hallux; the fourth branch anastomoses with a direct continuation of the long saphenous, and both innervate the medial aspect of the foot.

The *deep peroneal, medial terminal division*, in either foot, retains its normal position with reference to the second digit, between it and the lateral hallux, with no branches extending to the medial hallux.

The *medial plantar* in either foot possesses two branches which are comparable to the typical single medial proper digital branch; these are for convenience designated the medial and lateral divisions of that branch. The medial division divides into three digital branches which supply the medial and lateral surfaces of the medial hallux and the medial surface of the lateral hallux. The lateral division terminates in two branches, the first of which anastomoses with the second one of the medial division to supply the lateral surface of the medial hallux. The second branch anastomoses with the third one of the medial division and supplies the medial surface of the lateral hallux.

#### DISCUSSION

Annandale classifies supernumerary digits in four groups: 1) deficient, loosely attached by a narrow stalk to the hand or foot or to another digit; 2) more completely developed, free distally and articulating with the head or sides of a metacarpal, metatarsal, or phalanx; 3) completely developed and separate, and, 4) intimately united, longitudinally, with another digit, and either

provided with a metacarpal or metatarsal of its own or articulating with the head of one common to it and another digit. The case described is to be referred to class 3, since each hallux is separate and completely developed, even to the extent of possessing a metatarsal of its own. That the two most medial digits of either foot are halluces is demonstrated particularly by their external features, bones, and muscles.

Concerning the factors underlying the production of extra digits, at least three explanations have been advanced. An older view accounts for the anomaly on the basis of reversion. But, as is indicated by the facts briefly outlined in the following extract from Prentiss ('06), the theory is untenable in accounting for hyperdactylism in man or other pentdactylous mammals.

We may assume that the primitive and typical mammalian foot was pentdactyl, in spite of Bardeleben's contention that the progenitors of the mammalia possessed not five, but seven digits. Bardeleben's assumption was based upon the observation that certain mammals, the whale, for example, have more than five digits; that among five-toed forms six and seven digits occasionally occur; and that in many species small cartilages are present on each side of the hand and foot. These cartilages Bardeleben regards as digital rudiments, and the occurrence of extra digits is explained by him as reversion, a 'turning back' through heredity, to ancestral conditions. Unfortunately, the facts do not support this beautiful theory. Paleontology tells us that the forerunners of the mammalia possessed only five toes. Embryology has shown that the sixth digit of the whale, and the cartilages which Bardeleben supposes to be digital rudiments, develop secondarily some time after the typical five digits have appeared. Finally, observations have proved that the extra digits which occur in polydactylism do not develop from Bardeleben's 'digital rudiments,' but originate in an entirely different manner. We may, therefore, assume that the primitive mammalian foot was pentdactyl, and this being so, the occurrence of six or seven digits on a foot normally five-toed can not be attributed to reversion, unless we assume with Albrecht that it is reversion to the many-rayed fins of the Elasmobranch fishes, an absurd supposition.

The second causal factor postulated is that of mechanical influences which distort the embryonic hand or foot. It is not unlikely that some cases of imperfectly developed supernumerary digits, especially if unilateral, might be the result of compression or constriction by amniotic bands. In fact, Ahlfeld (cited by Prentiss) "has observed that digital duplications may



be caused 'in utero' by pressure from the thread-like growths of the amnion." The very existence of a family history of hyperdactylism, present in most cases, would argue against the operation of such a factor, as would the usual bilateral occurrence (Broman) of the duplication and the much more frequent involvement of the first and fifth digits rather than the intervening ones (Prentiss). Still more conclusive argument against a general postulation of mechanical factors is furnished in the existence of hyperdactylous identical twins. Stockard presents a case of identical twins in which both twins possess apparently the same type and degree of hyperdactylism—six digits on each hand and foot. In the same connection, Danforth describes the left hands of a pair of twins, possibly identical, each having a supernumerary fifth digit which is small and imperfectly developed.

We are led, then, to the probably almost invariable source of hyperdactylism, namely, an intrinsic factor or complex of factors, non-atavistic in nature. The arguments set forth above as opposed to mechanical induction favor not only the assumption of intrinsic factors, but also possibly an almost universal operation of potentialities transmitted by the germplasm. However, hyperdactylism occurs in monsters (Gräfenberg) as well as in individuals who are otherwise normal, the genesis of such monsters being explained by Stockard on the basis of developmental arrest. In these cases it seems not unreasonable to assume that causative factors which are non-germinal, but which operate intrinsically in modifying the normal rate of development, may bring about the condition of hyperdactylism coexistent with deformities which are unquestionably non-germinal in origin.

Prentiss repeatedly states that the germinal factor is not productive of invariable particular duplications, but rather is only a tendency toward duplication, exhibiting itself in successive generations as variable types and degrees of hyperdactylism or even merely as increased size of the first or fifth digits. Both Broman and T. Lewis note the frequent association of hyperdactylism and syndactylism in the same individual; Prentiss ('10) describes a case of hyperdactylism associated with deficiency

of the frontonasal process, and a legion of cases of heritable developmental defects coexistent with hyperdactylism occur in the literature. Then is it not warrantable to conclude that hyperdactylism of germinal origin is the immediate result of an instability in the autonomous control of developmental processes, the heritable factor being the instability and not the particular duplication? There seems to be a parallelism in the results of this instability and defects induced by experimental modification of developmental rate. Stockard concludes that various types of experimentally produced defects may be obtained by identical treatment of the embryos, and, further, that the type of defect is dependent upon the time at which developmental arrest is induced by the treatment. If such a parallelism does exist, the type and degree of hyperdactylism may be dependent upon the period at which germinal instability comes into play. On the other hand, if there is no correspondence in the action of experimental arrest and germinal instability, the degree of instability may, conceivably, be measured by the type and degree of duplication. In either case, it is apparent that a certain amount of selectivity exists, in that the first and fifth digits are most frequently affected. Prentiss points out that these digits are in the process of modification and regression, respectively; he suggests that these digits are thus more unstable, more plastic, and hence would be the most common sites of variation.

Hyperdactylism in the case described is assumed to have been produced by germinal factors. It is not believed that there was provision in the parental germ cells for both halluces in toto, or, if the foregoing arguments hold, even for the particular duplication. Rather, the immediate cause is presumed to have been an instability, transmitted germinally, perhaps in the form of a modification in rate of development which occurred in very early embryonic development. From the anatomical findings it is concluded that this instability resulted in a complete duplication of the germinal determinants for hallucal epidermis, hallucal phalanges, and first metatarsal. Further, it is concluded that the same instability need not have directly influenced muscles, vessels, and nerves, that the adaptation of these structures

to the doubled hallucal environment could have been secondarily induced.

Conclusions drawn from observations on individual systems are summarized in the following paragraphs.

Friction ridge patterns of the sole show no variations concerned with the hyperdactylous condition. By virtue of its position, the medial hallux has appropriated the hallucal pattern. The pattern itself shows no cleavage, duplication or even attempted duplication. In the absence of any disturbance within the hallucal pattern, it seems that there must have been no germinal provision for accessory hallucal patterns correlated with the duplication. In embryonic development the physical separation of the hallucal epidermal area into two isolated digital areas must have been preceded or accompanied by readaptation of the epidermal determinants. Determinants for general skin of the hallux, its nail and digital friction ridge patterns must have completely reproduced themselves. Their reproduction could have been incited through the primary action of the developmental instability (arrest?), or secondarily through doubling of the hallucal environment, but unlikely by a direct germinal provision for two hallucal digital epidermal areas, the unmodified hallucal pattern being contraindicative.

Doubled sets of bones, structurally like those of the normal hallux and its metatarsal, must be, developmentally, the result of a qualitatively complete duplication of the determinants for bones of the single hallux and first metatarsal. The time at which such duplication occurred and even the sequence of ontogenetic events following it are a matter only of conjecture. Potentially, two sets of bones may have been present long before they were evident structurally, the reproduction of determinants for bones having been an early and direct effect of the developmental instability. Once formed, the hallucal phalanges and first metatarsals seem to have served as cores or points of reference about which other hallucal structures were developed. Bardeen ('10) describes the normal development of the foot bones. Their first appearance is in the form of a foot plate, a condensation of the formerly more diffuse mesenchyme in the

distal portion of the posterior limb bud appearing during the fifth week. Toward the end of the fifth week, five localized areas of still greater condensation appear within it, the digital rays. During the sixth and seventh weeks, each digital ray segments transversely into, first, a metatarsal, and then the phalanges of a digit. Coincidentally, anlagen of the separate tarsal bones appear within the proximal portion of the foot plate, becoming more distinctly outlined toward the end of the second month. In our case the complete development of individual bones would point to an extremely early appearance of the supernumerary toes. Perhaps when first condensed within the foot mesenchyme the foot plate may have been wider than normal, its most medial region bearing tissue for the development of two metatarsals instead of one and two sets of hallucal phalanges. With the condensation of digital rays, six instead of five may have been formed, each of the two most medial carrying the germinal nature of the typical most medial one. Or, perhaps five digital rays appeared within the foot plate, and the most medial one was cleft longitudinally into two. It seems unlikely that a cleavage much later than this could be accountable for the well-developed bones met with in both feet. The duplication apparently does not invade the region of the tarsus. However, the accessory bone of the left foot may be the result of an abortive duplication of the first or second cuneiform, which in the right foot was secondarily ankylosed with the lateral first metatarsal. More likely, the accessory bone represents the epiphysis of the lateral first metatarsal, isolated in the left foot and normally fused with the shaft in the right.

The absence of a germinal provision for muscles is inferred from the absence of interossei between the two first metatarsals, the failure to produce any new muscles and the lack of adaptation of some muscles to both halluces. As a consequence of the last two deficiencies, the halluces of either foot are provided with an unequally distributed musculature. In the right foot the abductor, adductor, and both bellies of the flexor hallucis brevis insert wholly upon the medial hallux. The same muscles are more evenly allotted to the two halluces of the left foot, where the

abductor, medial belly of the flexor hallucis brevis, and only a very minor portion of the adductor insert upon the medial hallux, leaving the major portion of the adductor and the entire lateral belly of the flexor hallucis brevis for the lateral hallux. The tendons of the long hallucal flexor and extensor are bifurcated and insert upon both halluces of either foot. The distribution of muscles is regarded as secondary, the variations described being induced through the relations of the developing muscles to the already doubled osseous environment. From Lewis' account of the embryology of the foot muscles ('10), it appears that isolation of individual muscles from the premuscle masses may be either coincident with or subsequent to the appearance of anlagen for the separate bony elements with which they later become related; but the selection of their definitive insertions naturally follows the differentiation of the bones upon which they insert. The developing bones may be considered as points of reference which govern attachments of muscles, their influence being specific for particular bones and for localized regions upon any one bone. Whether the influence is determined through germinal inheritance or is the result of the recognized interdependence of structures in development, or both, a definite dynamic nature may be attributed to the developing bones. Muscle attachments in the feet described are believed to be the result of operation of such dynamic factors, variations concerned with hyperdactylism being secondarily produced through doubling of the bones. Assuming that each of the two halluces carries a full complement of the dynamic factors governing insertions of individual hallucal muscles upon particular areas of their bones, as is indicated by normal areas of attachment and typical morphology of the bones themselves, it is inferred that each hallux can control attachments of those muscles which are environmentally related to it, rather than to the other hallux. That the two halluces of each foot exerted approximately equal control of those muscles related to their dorsal and plantar surfaces is evidenced by bifurcation of the long extensor and flexor tendons. On the right side, the insertion upon the lateral hallux of a slip from the flexor digitorum brevis suggests the environmental

displacement of this hallux. This accessory slip and a similar one to the medial hallux from the flexor digitorum longus perhaps are the consequence of the unnatural expansion of their flattened embryonic tendon plates, due to increased width of the foot as a whole, with subsequent formation of supernumerary tendons. In the right foot the medial hallux more nearly assumes the nature of a normal hallux, with regard to musculature, and thus is assumed to have been in an environment approaching the normal. Of the four halluces concerned, the lateral hallux of this foot appears to be farthest removed from a hallucal environment while in the left foot the two halluces seem to be complementary with reference to the medial and lateral aspects of the normal hallux.

Since arterial variations occur, not in the courses of main trunks, but in the size, number, and distribution of terminal branches, it is inferred that they are the result of environmental relationships in development rather than of any germinal correlation between hyperdactylism and the differentiation of vessels. Senior describes the formation of dorsal and plantar plexuses within the mesenchyme of the foot, from which are derived all the arteries on the dorsum of the foot, the plantar arch and all the terminal branches on the distal portion of the sole (excepting the trunks of the lateral and medial plantar arteries). The three trunks, medial plantar, dorsalis pedis (resolved within the dorsal plexus), and lateral plantar, establish their permanent connections in the order named. While no emphasis is placed upon the ascendancy of the deep plantar and medial plantar in supplying the region of duplication, the fact is at least suggestive of a permanent dominance of those vessels which first establish their permanent connections in a region characterized by an abnormally large mass of tissue.

There is no involvement in the courses and fundamental relations of the nerve trunks. Thus the medial terminal division of the deep peroneal retains its typical relation and is not influenced by the medial hallux. This relation recalls the insertions of hallucal muscles as they obtain in the left foot. Branching of the medial dorsal cutaneous and medial plantar nerves is

suggestive of the bifurcated extensor and flexor tendons to the two halluces. The complex interrelation between the medial and lateral divisions of the medial proper digital may be the result of retained regionally specific dynamic influences of the normal single hallux. The combined cross-section areas of the two hallucal branches of the medial dorsal cutaneous are approximately twice that of the normal single branch; this relation holds also for the two branches of the medial plantar which supply the halluces. Unfortunately, no counts of the contained nerve fibers could be made, to determine whether the increased volume is due to excess in number of nerve fibers or amount of connective tissue. Even though a correlation between number of spinal ganglion cell bodies and increased area to be supplied does exist, it seems hardly necessary that germinal factors provided the mechanism for correlation. Occurring early, the duplication of the hallucal sensory area might still influence the proliferation of ganglion cells.

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Resumen por el autor, Albert Kuntz.

Un caso de preñez anormal con retención de los fetos muertos en el conejo.

Una coneja preñada exhibía en la cavidad peritoneal dos fetos muertos encapsulados a término y en el útero dos fetos normales vivos de 18 mm. de longitud. Los tejidos de los fetos muertos aparecían necróticos pero bien conservados. Las membranas coriónica y amniótica faltaban, excepto en el área placentaria. Las cápsulas consistían en membranas delgadas de tejido conectivo viable con abundante irrigación sanguínea, si bien su inserción en cualquiera de las estructuras intraabdominales era muy ligera.

Translation by José F. Nonidez  
Cornell Medical College, New York



## A CASE OF ABDOMINAL PREGNANCY WITH RETENTION OF DEAD FETUSES IN THE RABBIT

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### ONE FIGURE

In the routine of laboratory work a female rabbit was found which, when the abdominal cavity was laid open, exhibited two encapsulated dead fetuses in the peritoneal cavity and a pregnant uterus containing two normal living fetuses 18 mm. in length. I am indebted to Dr. M. S. Fleisher for this material.

The literature of obstetrics and gynecology contains the reports of not a few cases of abdominal pregnancy in the human species. In some of these cases a dead fetus was carried in the peritoneal cavity for a relatively long time following the normal period of gestation. Barring infection or accident in such cases, the fetal tissue slowly undergoes absorption. A few cases of abdominal pregnancy in animals have also been reported.

In the majority of the reported cases of abdominal pregnancy the fetal envelope and placenta, in cases examined early, or the capsule containing the dead fetus, in cases examined late, showed extensive and firm attachment to the peritoneum or mesentery. Not uncommonly, one of the ovaries was involved in the attachment. When the present case came into my hands the encapsulated fetuses were lying unattached in the peritoneal cavity. The laboratory assistants who had opened the abdominal cavity and removed the fetuses insisted that they had broken no attachments of the capsules to any intra-abdominal structure. Furthermore, careful inspection of the capsules revealed no areas of attachment.

The encapsulated dead fetuses, the ovaries, the uterus, and the living fetuses contained in it were fixed and preserved in 10 per

cent formalin and subjected to further study. The ovaries proved to be normal in all respects, containing both corpora lutea and corpora albicantia. The fallopian tubes also were normal. The presence of normal living fetuses in the uterus is of little importance except as an indication that the encapsulated fetuses were carried as dead bodies in the abdominal cavity for some time. There is no means at hand of determining how much time elapsed between the successive pregnancies. Encapsulated with each dead fetus was a mass of dead placental tissue. The capsule was closely applied to the fetus and the placental mass and presented the appearance of a thin connective tissue membrane. One of the encapsulated fetuses is illustrated photographically in the accompanying figure (fig. 1).

Upon microscopic examination the fetal tissues were found to be necrotic, but still sufficiently well preserved to admit of reasonably good staining. The skin was intact and well covered with hair. Sections through the capsule and the underlying skin revealed only remnants of amnionic or chorionic tissue. Obviously, these tissues were almost completely absorbed except in the placental area. The capsule consists of a thin connective-tissue membrane containing numerous small blood vessels. It does not blend with the placental mass, but is continuous over the surface of the latter and is of the same character in this area as in areas in which it lies in contact with the fetal integument. The capsular tissue was perfectly fixed and reacted to the stain like freshly killed connective tissue. It does not present the histological appearance of the mesodermal portions of the fetal membranes, but rather that of viable connective tissue. Therefore, the capsule is not of embryonic but of maternal origin. Inasmuch as the capsule consisted of living connective tissue with an abundant vascular supply, we must conclude that it was attached to the peritoneum or mesentery at some point, although the attachment was so slight that neither was it observed during the postmortem examination, nor was the area involved detected on later inspection of the capsule.

As indicated above, the ovaries and fallopian tubes were normal in all respects. No scars or other evidence of tubal or ovarian

pregnancy which might have ruptured or discharged into the peritoneal cavity could be detected. The possibility of primary abdominal pregnancy cannot be precluded. However, the absence of evidence of some other type of ectopic pregnancy in this case does not warrant the conclusion that the ova were extruded into the peritoneal cavity before they had secured implantation sites elsewhere. According to the modern view as

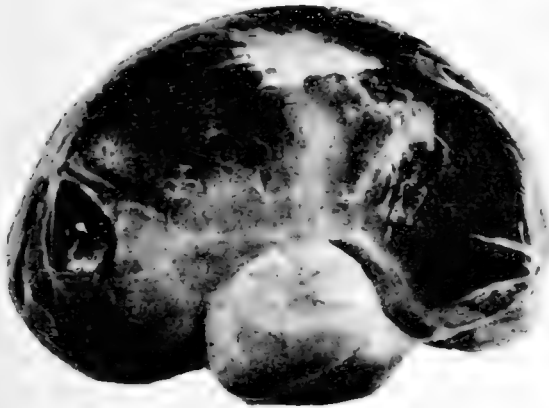


Fig. 1 Photographic illustration of encapsulated dead fetus

set forth by Kelly,<sup>1</sup> Williams,<sup>2</sup> and others, the theoretical possibility of primary abdominal pregnancy is admitted, but conclusive evidence that it actually occurs is not forthcoming. Conclusive proof of primary abdominal pregnancy in any case in which gestation is well advanced is obviously impossible. The facts in the present case are not incompatible with the theory that the ova were discharged into the peritoneal cavity after gestation was initiated.

<sup>1</sup> Kelly. Amer. Textbook of Obstetrics. 1895.

<sup>2</sup> Williams. Obstetrics. 1912.

## BOOKS RECEIVED

*(Continued from first page)*

AN INTRODUCTION TO ZOOLOGY by C. H. O'Donohue, D.Sc., F.Z.S., Professor of Zoology, University of Manitoba, 502 pages, illustrated. New York: D. Appleton and Company, 1921. "The object of this volume is to provide a text-book for the zoological position of the syllabus in Biology for the First Examination for Medical Degrees of the University of London, and the First Examination for the Conjoint Examining Board in England of the Royal College of Physicians of London and the Royal College of Surgeons of England. It is hoped that it will also prove useful to students preparing for similar examinations, and for those who are taking classes, like the pre-medical courses in American Universities, requiring a knowledge more particularly of Vertebrate Zoology." *From the Preface.*

THE ANATOMY OF THE NERVOUS SYSTEM from the standpoint of development and function, by Stephen W. Ranson, M.D., Ph.D., Professor of Anatomy in Northwestern University Medical School, Chicago. Octavo volume, 395 pages, 260 illustrations, some of them in colors. Philadelphia and London: W. B. Saunders Company, 1920. \$6.50 net. "The anatomy of the nervous system has been presented from the dynamic rather than the static point of view. Structural details become interesting when their functional significance is made known. During the past twenty years very considerable additions have been made to the science of neurology, and the more important of these have been included in the text."

SURGICAL ANATOMY, by William Francis Campbell, M.D., Surgeon-in-Chief at Trinity Hospital, Brooklyn, N. Y.; sometime professor of Anatomy and Professor of Surgery Island College Hospital. Third Edition, Revised. 681 pages, 325 illustrations. Philadelphia and London: W. B. Saunders Company, 1921. Cloth \$6.00 net. "The students interest in anatomy is vitalized only as the anatomic facts are correlated with those practical problems with which he is confronted as a practitioner of medicine."

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Resumen por el autor, G. J. Noback.

Métodos simples para la correlación de las longitudes occipucio-rabadilla y occipucio-talón del feto humano.

El autor presenta una fórmula que hace posible la utilización de datos sobre el feto humano en términos de la longitud desde el occipucio a la rabadilla (altura del ejemplar sentado) la cual se desea comparar o combinar con datos semejantes expresados en la longitud desde el occipucio a los talones (altura total o altura del ejemplar en pié). La fórmula mencionada es la siguiente:

$$CH = \frac{3 CR - 3}{2}$$

en la cual C corresponde a occipucio, H a talones y R a rabadilla.

Para determinar la longitud desde el occipucio a la rabadilla cuando se conoce la del occipucio a los talones se puede emplear una segunda fórmula:

$$CR = \frac{2 CH + 3}{2}$$

Para determinar la exactitud de las fórmulas precedentes se han empleado las observaciones de varios investigadores que han publicado datos basados en ambas longitudes.

## SIMPLE METHODS OF CORRELATING CROWN-RUMP AND CROWN-HEEL LENGTHS OF THE HUMAN FETUS<sup>1</sup>

G. J. NOBACK

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### ONE FIGURE

Investigators in prenatal anatomy and development report their findings in one of three ways as regards length of the individuals studied. Some writers use both crown-rump and crown-heel length (Michaelis, '06; Mall, '10). Others use only the crown-rump (Streeter, '20), and some use the crown-heel alone (Meyer, '15; Scammon, '19; Noback, '21). Work based on one of these measurements alone is obviously difficult to compare with work based upon the other.

The relation between the crown-rump and the crown-heel lengths during fetal life is practically constant. The formulae here presented further demonstrate this fact.

The crown-heel length of the human fetus is equal to the crown-rump length (in millimeters) minus one, plus one-half of this amount. The following formula makes possible the utilization of data reported in crown-rump length which is to be compared or combined with data based upon the crown-heel length alone.

$$CH = \frac{3CR - 3}{2}$$

Illustration: The known crown-rump length is 95 mm. What is the corresponding crown-heel length?

$$CH = \frac{3 \times 95 - 3}{2}$$

i.e., the crown-heel length is 141 mm.

<sup>1</sup> The formulae in this report were demonstrated at the thirty-seventh session of the American Association of Anatomists, March, 1921.

The second formula may be used to determine the crown-rump length when the crown-heel length is known. It is expressed as follows:

$$CR = \frac{2CH + 3}{3}$$

Illustration: The known crown-heel length is 141 mm. What is the corresponding crown-rump length?

$$CR = \frac{2 \times 141 + 3}{3}$$

i.e., the crown-rump length is 95 mm.

In order to determine to what extent measurements made by different observers would deviate from the formulae, I have plotted them on ordinate paper against the curve obtained by use of the formulae. The crown-rump lengths are represented by the abscissas and the crown-heel lengths by the ordinates. The accompanying plate shows the formulae curve as a straight line, and it is readily seen that the measurements made by the several observers are distributed quite evenly about it.<sup>2</sup>

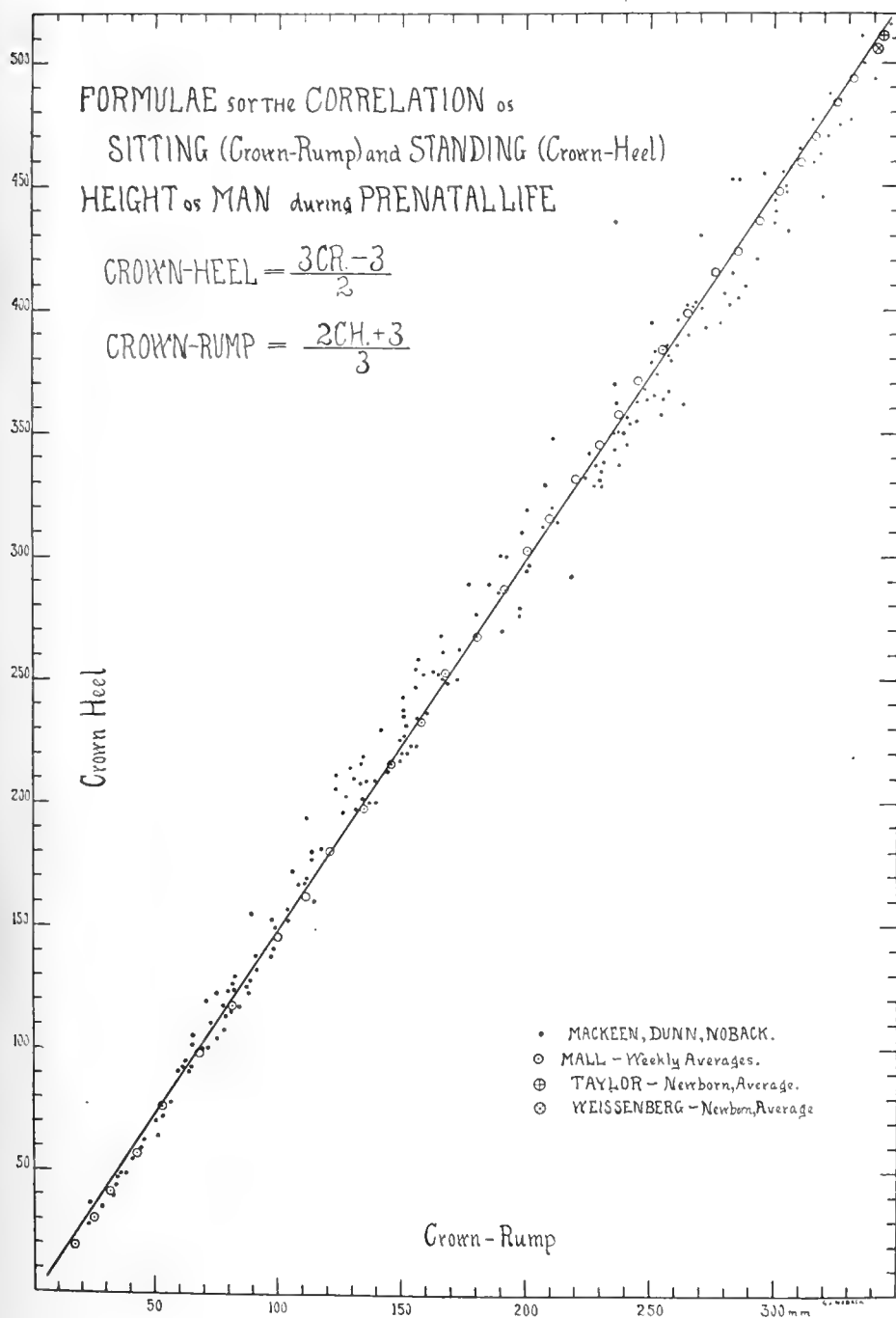
The following table shows the extent of deviation from the lengths derived by formula in the data of Mall, MacKeen, and Michaelis.

	AVERAGE DEVIATION	PERCENT- AGE DEVIATION
Mall (weekly averages of 1000 cases).....	2.4	0.7
MacKeen (161 cases).....	9.5	3.6
Michaelis (weekly averages from 14 weeks to birth).....	11.5	3.3

Two sets of data on measurements of the newborn infant are also plotted against the curve. These observations were made by Taylor '19 and by Weissenberg '06. The averages of their measurements vary but a trifle from the curve derived by the formulae.

<sup>2</sup> Besides data of Mall and of Michaelis I have used measurements made in the Institute of Anatomy, University of Minnesota, by Helen Adams MacKeen and by Halbert Dunn. The work of the latter two investigators will appear in due course.





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Resumen por el autor, Leslie B. Arey.

Prueba directa del origen monozigótico de los gemelos humanos idénticos.

Los gemelos humanos idénticos o mellizos homólogos se originan a expensas de un solo óvulo, conforme demuestra el autor por primera vez en el presente trabajo. El saco vitelino humano es un vestigio innecesario para el crecimiento o la diferenciación (incluso la vasculogénesis). La doctrina del origen local de la vasculogénesis está soportada por los hechos, dentro de los límites de la posibilidad experimental, en los mamíferos.

Translation by José F. Nonidez  
Cornell Medical College, New York

## DIRECT PROOF OF THE MONOZYGOTIC ORIGIN OF HUMAN IDENTICAL TWINS<sup>1</sup>

LESLIE B. AREY

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ONE PLATE (SIX FIGURES)

The commonest seat of ectopic pregnancy is the uterine tube. When such tubal gestations are plural, they may be either unilateral or bilateral in position. The former type, that is, the presence of more than one fetus in the same tube, is somewhat the rarer; a careful examination of the literature ('22 a) has yielded thirty-eight positive or authentic cases, eight probable or presumptive cases, and four possible but doubtful cases. To the positive list I have there added two new cases of mono-chorial twins, each unique of its kind. Detailed descriptions of these specimens, summaries of all the other cases, together with the general conclusions drawn from an analysis of the anatomical and clinical data afforded, may be found elsewhere ('22 a, '22 b, '22 c). This note is confined to the presentation of certain features of peculiar embryological interest and significance.

The first of the two new specimens,<sup>2</sup> consisted of a single chorionic sac which contained twin embryos, each 12.3 mm. long (fig. 1). There is a common yolk sac from which distinct yolk stalks arise near together and pass to their respective umbilical cords. The cut stumps of these yolk stalks and their separate origin from the sac are clearly shown in figures 2 and 3.

<sup>1</sup> Contribution no. 90.

<sup>2</sup> This twin specimen belongs in the collection of the Carnegie Institution, where it is listed as no. 1126. Acknowledgment is due the director, Dr. George L. Streeter, for permission to describe it. He has also furnished the accompanying photographs (figs. 1 to 3).

There has been no real doubt that human homologous or identical twins come from the division of a single egg. Many facts point incontestably to this conclusion; among such are their morphological duplicity, their occasional conjoining as 'double monsters,' and a common chorion.<sup>3</sup> Direct corroboration is furnished by the known manner of quadruplet development in the Texas armadillo, as well as by experiments and observations upon the lower vertebrates and invertebrates. Yet this specimen of human twins demonstrates for the first time their actual origin from a single yolk sac, and hence from a single ovum. Schwalbe's ('06) well-known reconstructions, based upon the Spee 1.54-mm. embryo, are purely hypothetical. Beside the present case, the only other illustrative specimens among mammals are the normal developmental stages of the Texas armadillo (Newman and Patterson, '10) and Assheton's ('98) single case of a sheep's blastodermic vesicle which bore twin germinal areas.

The second new twin specimen is in some respects even more interesting. Within the single chorion were twin embryos of 11.5 and 12 mm. (fig. 4). Each had its individual umbilical cord; these were attached to the chorionic wall, a quadrant's distance apart. Adherent to the amnion of one embryo (at the left in fig. 4) was a yolk sac of normal size; its cut stump may be seen in the photograph. The other embryo has no yolk sac. To make certain of this, both umbilical cords were sectioned throughout their lengths. That of the embryo at the left was typical for this age (fig. 5). The yolk stalk with its vitelline vessels lies in a coelomic extension; the level of the section here reproduced is too far distad to include the allantois. The cord of the embryo at the right is without microscopic trace of yolk sac or stalk, although the remaining structures are normal (fig. 6).

Certain deductions may also be made from this specimen. Mammalian monozygotic twins do not arise by the separation of early blastomeres, as too often has been assumed; on the

<sup>3</sup> In another publication ('22 c) it will be shown that a common chorion is not infallible proof of the monozygotic origin of human twins.

contrary, the common chorion which all twins of this sort possess proves at once that separation must be subsequent to the period when the cleavage group is differentiated into an inner cell mass and an outer shell of trophoctoderm. That identical monochorionic twins arise by the fusion of separate ova need not seriously be considered, although it is apparent that some double ovum (fraternal) twins do become monochorial, in this way ('22 c). There is considerable evidence to support the belief that the twinning impulse occurs relatively late, at about the moment of gastrulation, which in mammals corresponds to the time of the formation of a primitive streak; the process, then, is simply one of double gastrulation. Studies on the development of the Texas armadillo (Newman and Patterson, '10) and Stockard's ('21) basic experiments and conclusions on the production of twins both substantiate this belief.

Exactly how determinate the mammalian ovum is in its development and how rigidly formative stuffs are localized are matters of speculation. The manner of fission into the two embryonic masses was presumably such that one received all, or essentially all, the cells destined to form a yolk sac; this again confirms the idea of a late moment for twinning when other cells had passed the stage of fundamental multiple potentialities.

The total absence of a yolk sac in one embryo which is otherwise normal in every way further demonstrates conclusively that this organ is not essential to the growth of an embryo or to the proper differentiation of its parts; indeed, the embryo in question is slightly larger than its twin, whereas both correspond closely in size to the norm for that menstrual age.

The embryo without a yolk sac is of further interest in relation to the ingrowth doctrine of vasculogenesis. In so far as such evidence is trustworthy, it supports rather the local-origin view, now generally acknowledged. That tiny vascular anlagen of yolk-sac ancestry actually existed and at an earlier moment helped form primitive vessels by extension into the embryo is, of course, conceivable; also the theoretical possibility of vascular ingrowths from the early anlagen in the body stalk are not excluded because of the characteristic and necessary nutri-

tive relations of the mammalian ovum. Fortunately, the fundamental truth of the local-origin doctrine rests on observations more rigorously controlled than this natural experiment which, however, was performed as perfectly as ever may be expected in a higher mammal.

In general, the yolk sac of man and the higher mammals appears to be an unnecessary vestige. In the earliest human embryos known, when it might be of real use, it is a simple entodermal sac containing masses of coagulum; growth to a conspicuous size is attained relatively late, long after adequate nutritional relations with the mother have been thoroughly established.

It is a great coincidence that both specimens of so rare a type should show features of such singular value and interest.

#### SUMMARY

Human identical or homologous twins for the first time are actually shown to originate from a single ovum.

The human yolk sac is a vestige unessential to growth or differentiation (including vasculogenesis).

The local-origin doctrine of vasculogenesis is supported within the limits of experimental possibility on mammals.

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## PLATE

## PLATE 1

### EXPLANATION OF PLATE

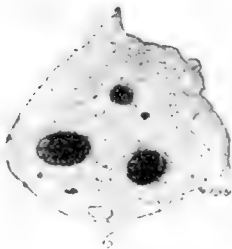
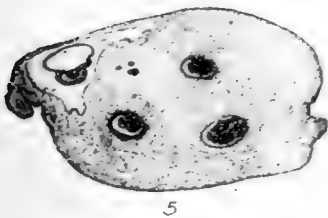
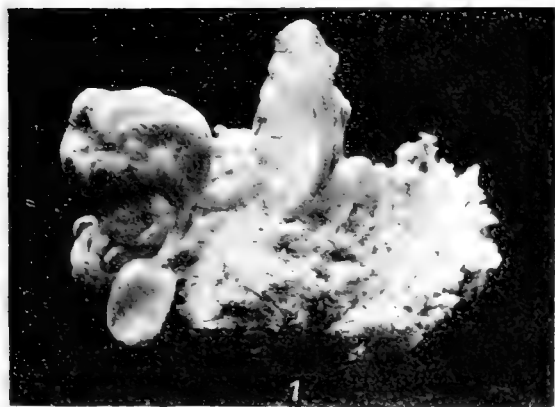
1 Photograph of 12.3-mm. human tubal twin embryos attached to a common yolk sac.  $\times 2.2$ .

2 and 3 Additional photographs of the common yolk sac shown in figure 1. The origin of the separate yolk stalks is apparent.  $\times 2.2$ .

4 Photograph of a pregnant human uterine tube. Part of the wall has been removed to show the twin embryos in place. The single yolk sac belongs to the 11.5-mm. embryo at the left. The 12-mm. embryo at the right lacks both yolk sac and stalk.  $\times 1.7$ .

5 Photomicrograph of a section through the umbilical cord of the embryo at the left in figure 4. The yolk stalk lies in a coelomic extension. The level is too far distad to include the allantois.  $\times 23$ .

6 Photomicrograph of a section through the umbilical cord of the embryo at the right in figure 4. There is no trace of a yolk stalk. The delicate tube below is the allantois.  $\times 23$ .



4

Resumen por el autor, Leslie B. Arey.

Fusión coriónica y gemelización aumentada en el oviducto humano.

Los datos que poseemos hasta el presente indican que la relación de los gemelos monocoriónicos y la de los dicoriónicos es unas quince veces mayor en el oviducto que en el útero. Los estados ilustrativos indican que esta desproporción de los ejemplares monocoriónicos resulta en parte de la fusión secundaria de los sacos coriónicos de los individuos dizigóticos. Por esta causa, en el caso del hombre, el término monocoriónico no debe usarse siempre para expresar el origen a expensas de un solo óvulo. Un factor todavía mas potente parece ser el aumento actual del impulso productor de gemelos. Su causa puede referirse más claramente a la inflamación tubal y sus resultados. Los mismos retardamientos y cesación del desarrollo que producen y siguen a la implantación tubal son responsables, respectivamente, de la gemelización excesiva y la malformación de los ejemplares obtenidos del oviducto.

Translation by José F. Nonidez  
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## CHORIONIC FUSION AND AUGMENTED TWINNING IN THE HUMAN TUBE<sup>1</sup>

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FOUR FIGURES

### INTRODUCTION

Monozygotic twins of the higher mammals are necessarily monochorial. It is likewise customary to accept a common chorion as proof that mammalian twins are identical or homologous, that is, monozygotic in origin. This, however, need not be an infallible criterion. One of the armadillos, *Dasypus villosus*, has usually two monochorial young at birth; Fernandez ('15) has shown that these embryos at first occupy individual chorionic vesicles which then progressively fuse until they appear as one (3.5-cm. stage). Fusion of twin chorions among ungulates is apparently common; Lillie ('17) has proved beyond doubt that only rarely does such union fail in twin cattle and that it also occurs in the sheep and hog. There are no other data known to me. Lillie expresses the following opinion (p. 400): "A number of mammalian groups could be at once excluded from consideration because the conditions of placentation are such as to prohibit chorionic fusion; in mammalian groups such as primates and many rodents in which the ovum becomes embedded in the uterine mucosa, there is, of course, an insuperable bar to early chorionic fusion." Nevertheless, in the human tube at least—and here, except spatially, the conditions of implantation are essentially identical with those in the uterus (Mall, '15)—indications of chorionic fusion occur. This truth became apparent during an analysis of the data accompanying the recorded cases of tubal twin pregnancy ('22 a). The supporting evidence will now be presented.

<sup>1</sup> Contribution no. 92.

## STATISTICAL EVIDENCE OF CHORIONIC FUSION

First, the subject is best approached statistically. A rigid examination of the literature has revealed forty cases of tubal twins that appear authentic ('22 a); in addition there are eight cases which may be termed probable or presumptive. Among the positive list thirty-one specimens are recorded as having a common chorion, although apparently four more should be included, whereas in three only was it double.<sup>2</sup> In the probable group there are six definite statements; four of the six cases have double chorions.<sup>3</sup> Combining both sets, the ratio becomes 33 (or possibly 37) : 7. To the list of double-ovum twins must be added those that develop bilaterally, one in each tube. There appear to be less than a dozen proved cases.<sup>4</sup> Hence the total records show that monochorial specimens occur twice as frequently as the dichorial cases.<sup>5</sup> Since, however, viable (uterine) identical twins are but one-seventh as frequent as the ordinary fraternal type, it follows that tubal monochorial twins are some fifteen times more common than might be expected if the tube were no more favorable than the uterus as a seat for twin production.

<sup>2</sup> In one of the three the evidence is not unequivocal for doubleness.

<sup>3</sup> This relatively high ratio of doubleness is explained largely by the fact that duplicity itself was one of the determining factors utilized in rejecting several inadequately established cases from the positive group.

<sup>4</sup> McDonald and Krieger ('13) collected the recorded cases of bilateral tubal pregnancy. They listed twenty-five proved cases, including both twin and successive specimens. Only in nine instances were both fetuses present and of the same age; in eight cases they were of different ages (including two with one member a mummy and one a lithopedion); two specimens had a fetus in one tube paired with a clot and villi in the other; in two cases there were clots with villi in both tubes. Twenty-seven additional cases were listed as 'probable but not proved'; as a matter of fact, in none of these instances was there positive anatomical evidence of bilateral gestation, either twin or successive; sixteen combined a fetus in one tube with a hematoma without villi in the other, while eleven were represented by hematomata in both tubes.

<sup>5</sup> In this computation that group of dichorial cases which combine a simultaneous tubal and uterine pregnancy was, of course, ignored, for it belongs properly to neither category. Furthermore, comparable tubal conditions which allow one member of such pairs to descend uninterruptedly doubtless subtract a proportionate number from the monozygotic tubal specimens.

In view of these numerical relations, the preponderance of monochorial tubal twins seems to offer *prima facie* evidence of some modifying factor or factors. What these are will next demand consideration.

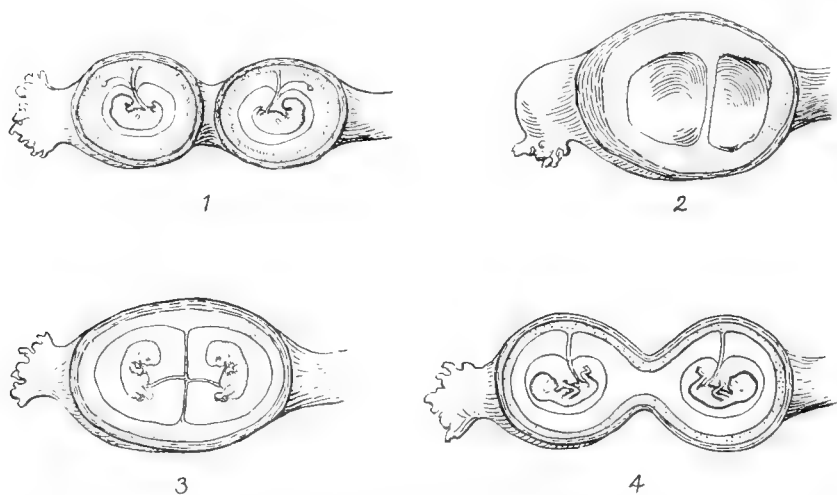
#### ANATOMICAL EVIDENCE OF CHORIONIC FUSION

The readiest explanation of the foregoing numerical disproportion is that separate ova have fused to produce pseudomonochorial twins. With this hypothesis in mind, the descriptions of all recorded cases of unilateral tubal twins were examined to ascertain if there is evidence of chorionic fusion in man. Briefly, such evidence does exist, as the following selected cases exemplify.

First in the series is Young's ('11) specimen, which comprises a tube with two dilatations; one represents a ruptured chorionic sac with its embryo still inside; the other sac was unruptured, entirely distinct from the first, and contained a twin embryo. Amann's ('08) case is apparently in the same category, and the clinical histories and anatomical descriptions recorded by Ferroni ('03), Saniter ('05), and Shauta ('05) make it probable that these also should be included. There is more doubt in the cases of Fenger ('91) and Salin ('95); they may be successive pregnancies. Figure 1 is a diagram of this preliminary condition based upon Young's specimen. Whether these particular ova ever would have fused had development continued or whether they were too far separated is unimportant; they typify the primary relation.

The next stage in fusion is illustrated by a tubal pregnancy described by Mall ('15) and Meyer ('20), listed in the Carnegie collection as no. 825 (fig. 2). Externally the tube bore a single swelling the size of a walnut, but section revealed two well-preserved chorionic vesicles, lying at about the same transverse level in the tube and flattened on the surfaces of mutual contact. The opposed chorionic faces had the appearance of a thin septum (Mall, pl. 9, figs. 4 and 5; Meyer, fig. 1), which proved to be double, partially separated by villi. Actual embryos and amnia had disappeared.

Pulcher ('05) has described a fusion still more advanced; it is a stage just prior to complete union (fig. 3). At the middle of the tube was a tumor the size of a fist. Its interior was divided by a thin translucent septum into two chambers, each of which contained a well-preserved 2-cm. embryo. Both umbilical cords were inserted near the center of the common septum. This partition was studied microscopically by sections; it is said to consist of a double layer of amnion. Nevertheless, since the umbilical cords pass to the septum, it must funda-



Figs. 1 to 4 Stages illustrating secondary chorionic fusion between dizygotic tubal embryos. Fig. 1, Young's specimen; fig. 2, Mall's specimen; fig. 3, Pulcher's specimen; fig. 4, Costa's specimen.

mentally represent a former double chorionic wall which had thinned and degenerated until it was no longer recognizable as such to the investigator.<sup>6</sup> During the growth of these ova, the nutritional mechanism necessarily became progressively incompetent. Under these conditions, the embryos should be underdeveloped; in accordance with this expectation, we find the

<sup>6</sup> Possibly the amnion was a constituent as well; each chamber is said to be lined with a 'smooth glistening membrane.' As this was the author's inaugural thesis, he presumably studied the specimen with care, although he failed properly to interpret the septum or the significance of the insertion of both umbilical cords in it.



menstrual histories indicating fetuses of twelve to fourteen weeks, when in reality they measure but 2 cm., which corresponds to seven weeks' normal development. It was a fortunate chance that the umbilical cords were inserted in the abutting chorionic walls; otherwise the specimen would have passed (as Pulcher believed) for twins with a common chorion and double amnion.

The final step in fusion is furnished by Costa ('07). Externally, the tube exhibited two dilatations, well separated and apparently distinct; internally, a neck-like constriction partially divided two communicating compartments, each of which contained a fetus, 5 and 5.3 cm., respectively (fig. 4). It is, of course, possible to interpret this as a single gestation sac secondarily constricted, but as it is a normal specimen, preceded by graded stages, fusion is more logical. The chances are greatly against the coincidence of single-ovum twins being so significantly spaced within a doubly dilated tube and chorion.<sup>7</sup>

Absolute proof of chorionic fusion waits upon the finding of male and female fetuses inside a common sac. Eventually such specimens may be confidently expected, especially since it is now possible to recognize sex in embryos as small as 15 mm. (Spaulding, '20).

In supplemental support of the principle of chorionic fusion, it is significant that two fetuses may occupy one gestation sac even when the history and anatomical relations point clearly to successive, rather than simultaneous pregnancies. Hence fusion, or inclusion, is certain. An illustrative case is that of Henricius and Kolster ('99), who attended a woman during two successive pregnancies without delivery on either occasion; at autopsy the left tube proved to be a single sac which contained a recent full-term fetus and a mass of fetal bones. A similar case is recorded by Racoviceanu and Bogdanovici ('04); the patient twice went to term without delivery; the right tube was a sac which contained a small, shrunken, but well-preserved fetus and an old lithopedion.

<sup>7</sup> Mall ('15) has described rare cases of degenerating ova without embryos which assume an hour-glass shape during dissolution. This process has no relation to Costa's specimen.

In passing, it should be mentioned that the amnia of twins also join readily. Twelve cases in the twin series specifically mention this point; in eight specimens the amnion was single.

#### AUGMENTED TWINNING IN THE TUBE

Although chorionic fusion may be invoked satisfactorily to reduce in part the preponderance of monochorial tubal twins, it is pertinent to inquire if the relative frequency of monochorial tubal twins may not be largely explained by an excessive production of monozygotic twins rather than by the fusion of the chorionic sacs of dizygotic individuals. In order to return an answer, it is necessary to consider both the cause of twinning and of tubal implantation.

Stockard ('21) has been able convincingly to reduce the primary cause of all non-hereditary abnormal development, including true (monozygotic) twinning, to a single factor—developmental inhibition or arrest; the exact type of deformity that results depends solely on the precise moment when the interruption occurs. Thus, twin formation results from a slowing of the developmental rate at the time of gastrulation, which in mammals corresponds to the period of the primitive streak. Such an arrest allows other potential embryonic axes on the blastoderm to assert themselves, whereas in normal development they are dominated and suppressed by the chief axis. Direct experimentation on fishes refers the cause of arrest to retarded oxidations. Occasional twinning in the chick and the customary quadruplet formation in the Texas armadillo lend strong support to this view. Both are subject to arrest and reduced oxygen supply—the chick by a lowering of the temperature when laid, the armadillo blastocyst by a quiescent period of several weeks in the uterus before it becomes implanted.

The direct application of this fundamental concept to abnormalities and twinning in the human tube follows logically when once the conditions attending tubal implantation are known. Mall ('15) studied the extensive Carnegie collection of embryos, in which many clinical histories accompany the specimens. He concluded that tubal pregnancy is associated with

preceding inflammatory changes, usually of long standing. It appears that such inflammatory disturbances may continue over long periods during which the inflammation is constantly too severe to permit the germinal products to unite or maintain development; later, however, the condition may abate sufficiently so that the ovum can develop, at least for a time, although it is still incapable of reaching the uterus.<sup>8</sup> Incidentally, it seems probable that if tubal pregnancy had not occurred in these cases when it did, the tube might soon have healed sufficiently to allow ordinary uterine gestation to proceed. The ovum fails to gain its normal destination in the uterus because of the impairment of the ciliated cells, as well as by kinkings of the tube through adhesions, by becoming trapped in epithelial diverticula of inflammatory origin or through adherent mucosal folds. "In short any change which delays the ovum in its progress will favor tubal pregnancy." An ovum taken up by the mucous membrane at the outer end of the tube may be delayed by the impaired ciliated cells until it is too large to pass the narrow end of the tube; it may wander into blind pockets or diverticula; more commonly still it is transported to the middle of the tube, where inflammation and follicular salpingitis are most common, and there become stranded because the ciliated cells can carry it no farther. Any of these delays, therefore, to which may be added the rarer hazards afforded by blind tubes, pocketed and double tubes, or delays due to migration from the opposite ovary, are the effective primary factors conditioning tubal implantation.

When the normal progress of the ovum is blocked in one of the ways designated it may then attack the tubal wall; in the meantime, however, it has attained a later stage of development than is customary at implantation. If, therefore, such delayed implantation and the establishment of tardy or inadequate oxygen relations (see below) should cause a developmental slowing at the critical moment for twinning, two embryonic axes would assert themselves as in the fish, chick, and armadillo, and mono-chorial twins result. This outline of events becomes a natural

<sup>8</sup> In another publication ('22 b) I have shown that this sterile period averages nearly six years.

inference when Stockard's discovery is applied to the known condition of the tube and ovum in tubal pregnancy. It helps account rationally for the preponderance of single-ovum twin pregnancies in the tube, which, by its greater susceptibility to prolonged and slowly clearing chronic inflammation, further explains why the uterus bears relatively fewer monozygotic twins.

The theory of Stockard is further supported in its human application by the demonstrable frequency of faulty implantation in tubal cases and its relation to malformation of the parts. Mall failed to find a typical decidua here, and hence its function in checking hemorrhage by forming a dam between the tips of the villi and the eroded mucosa is foregone. There result numerous hemorrhages which form old clots between the villi. "Even the best of specimens frequently show such extensive hemorrhages around the chorion and such marked degeneration of the villi that it is a wonder that the ovum continues to grow normally." Checks, permanent or temporary, while the chorion is struggling to overcome natural deficiencies in its nidus are sufficient to account both for excessive twinning, if the delay prior to implantation be not sufficient, and for the observed double frequency of anomalies in these specimens over the uterine group.<sup>9</sup>

Mall held that the primary causative factor of these pathological embryos is faulty implantation which prevents an adequate transfer of nutriment. Stockard agrees with the primary factor, but from his experimentation is forced to attribute the proximal cause to inadequate oxygenation.

#### SUMMARY

The available data indicate that the ratio of monochorial to dichorial tubal twins is about fifteen times greater in the tube than in the uterus.

Illustrative stages indicate that this disproportion of monochorial specimens results in part from the secondary fusion of

<sup>9</sup> Mall ('17) studied the anomalies of 1000 embryos and records that tubal monsters are twice as common as those in the uterine group.

the chorionic sacs of dizygotic individuals. Hence for man the term 'monochorial' need not always connote a single-ovum origin.

A still more potent factor appears to be an actual augmentation of the twinning impulse. Its cause is most clearly referable to tubal inflammation and its sequelae. The same delays and arrests which cause and follow tubal implantation are respectively responsible for the excessive twinning and malformation of tubal specimens.

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Resumen por el autor, C. E. Tharaldsen.

Una estufa de inclusiones en parafina para uso individual.

El presente trabajo es una descripción de la construcción y modo de funcionar de una estufa para inclusiones en parafina, adaptada para colocarla en una mesa de uso individual, la cual incluye facilidades para infiltración, inclusión, extensión de los cortes sobre porta-objetos, para secar, matar bajo la acción del calor, fijar y teñir en caliente, combinadas todas ellas en un aparato compacto dispuesto de tal modo que los objetos reciben el calor desde la parte superior del aparato y están situados convenientemente para el técnico. Las unidades que suministran calor consisten en dos bombillas eléctricas de 16 bujías, que pueden operarse por separado mediante una resistencia de cinco unidades, por medio del cual puede regularse la temperatura con rapidez y exactitud.

Translation by José F. Nonidez  
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## A PARAFFIN OVEN FOR INDIVIDUAL USE

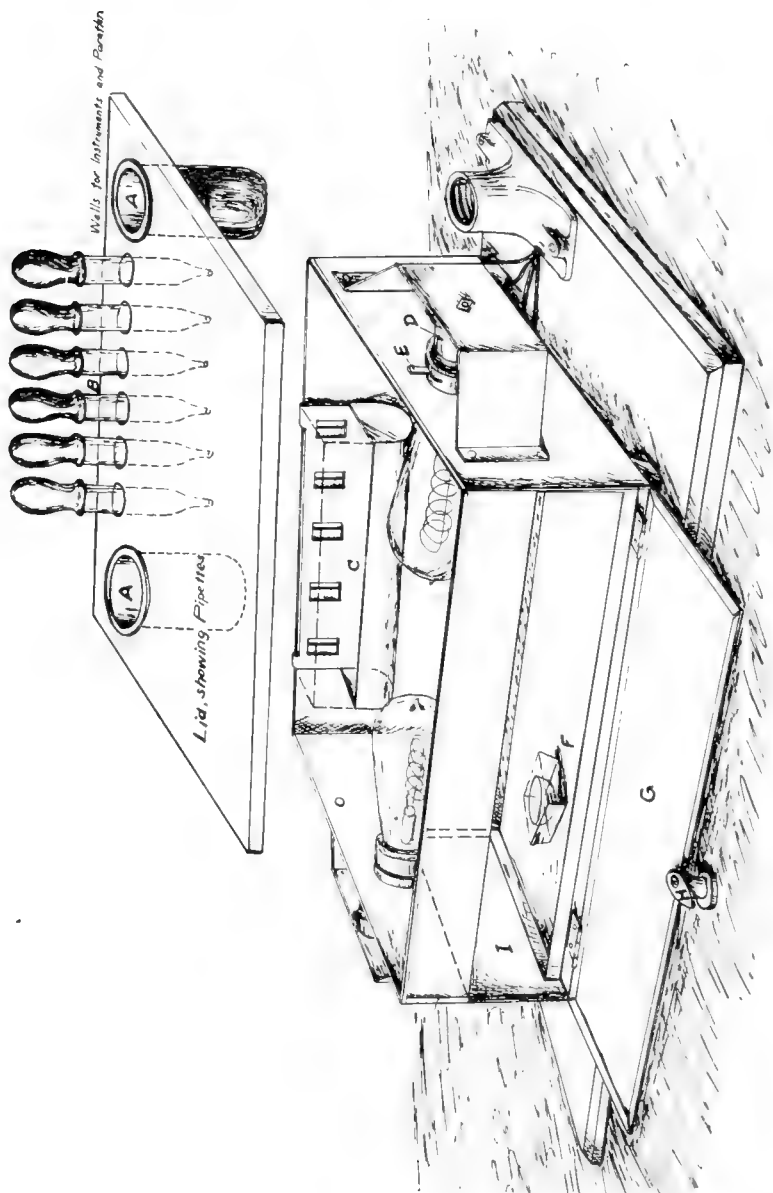
C. E. THARALDSEN

*Zoological Laboratory, Northwestern University*

### ONE FIGURE

That the inefficiency of our common types of paraffin ovens has been long felt is evidenced by the many home-made contrivances which have been devised to overcome this or that difficulty. Necessity, that prolific mother of invention, has indeed been bountiful, for what microtommist does not have his own contrivance for the paraffin process! A survey of such of these as have been described shows that the trend has been away from the large and elaborate ovens, towards the small, compact device that can be placed on the individual desk, where it is instantly available for use. The danger of fire from rheostats, gas and oil burners has long since led to the common usage of electricity as a safer source of heat, at the same time making possible the additional advantage of illumination. The time-consuming process of regulating water-baths has caused many to discard these in favor of a direct heat source from above the objects, thus securing a more rapid and uniform distribution of heat by radiation. The inconvenience and expense of several pieces of apparatus adapted to some particular phase of the paraffin process, i.e., infiltration, imbedding, spreading sections, drying slides, hot fixation, and staining, has led to repeated attempts to incorporate all these features in one machine.

It is one of these home-made contrivances which I have been requested to describe. This paraffin oven is one which for several years has been slowly evolutionizing in the Zoological Laboratory of Columbia University. The last model, which was designed by Young, formed the nucleus about which the present oven was constructed. This model, constructed in the Zoological Laboratory of Northwestern University, has now been installed



in both of the above-named institutions as well as several others, as an oven for individual use. In its construction it embraces all of the above-mentioned principles in one compact apparatus, and in these institutions its efficiency has been demonstrated by thorough trial. Steps are being taken to have it patented, not for the purpose of exploitation, but rather to protect it from such, that it may be available at a minimum cost.

The oven consists of a rectangular metal box of firm construction, measuring 10"  $\times$  5"  $\times$  6", mounted on a wooden base. The heating unit consists of two carbon filament 16-c.p. electric lights, each mounted in a five-unit reductor control (*D*) which is regulated by means of a lever (*E*). These reductor units and levers for their control are located at the upper part of either end of the oven so that they are conveniently placed for regulation, and the whole is firmly supported and protected by guard plates. On the floor of the oven a movable tray (*F*) is fitted for containers of paraffin, etc. In this way an even distribution of heat is secured on the objects by radiation from above, and at the same time they are illuminated conveniently for manipulation. By means of levers the temperature can be regulated to suit the conditions demanded by the nature of the work, from that of slight warmth for drying purposes to beyond the melting-point of the hardest paraffin. Expensive and inconvenient thermostats, rheostats, and thermometers have been eliminated from the equipment as unnecessary.

The front of the oven consists of a door (*G*) which opens downward, the knob (*H*) of which is of such a length that the door when open lies on a level with the floor of the oven. This surface thus serves the purpose of a shelf on which the tray for paraffin containers can be moved out or in for greater convenience in transferring tissues, orienting objects, or for imbedding. It also serves as a secondary temperature control in short time infiltration for finer cytological work. On either end wall of the oven is located a metallic wing (*I*), which can be pulled out even with the tray on the surface of the door. This is for the purpose of protecting the paraffin from side drafts, should this be found necessary. For longer infiltration methods, such as

are required by some plant and larger pieces of animal tissues, the tray is left in place and the door closed, after which the temperature is regulated.

The top of the oven consists of a removable lid, in the rear corners of which are suspended two wells (*A A*), one for containing the stock paraffin and the other for holding needles and such other instruments as are used in work with paraffin. Thus the paraffin and instruments are conveniently accessible, and both, because of their location to the rear of the heating units, are kept at a temperature most favorable for such work.

Between these two wells are six holes (*B*) in which are suspended paraffin pipettes. These pipettes hang down into a trough (*C*) which serves the dual purpose of collecting paraffin drippings and of regulating the temperature of the pipettes for the paraffin work. For the latter purpose a series of vents are arranged on the rear wall of the trough which correspond to a similar set on the rear wall of the oven. The temperature of the interior of the trough is controlled by sliding the one series of vents over the other, thus regulating the size of the aperture.

The front two-thirds of the lid is flat and without obstructions. Its temperature can be regulated from high to low uniformly, or one side can be maintained at a higher temperature than the other by means of the levers at either end. This surface thus serves as a very efficient warm plate for the spreading of sections on slides without danger of melting the paraffin. It may also be used for hot staining on the slide without danger of boiling or burning, such conditions as are required by the carbol-fuchsin smear preparations of sputum or the Benda and Bensley methods.

For the rapid drying of sections on slides after spreading, the process is conveniently accomplished by placing the slides on cardboard, which is then placed on the tray, after which the door is closed to protect from dust, and the temperature is then regulated to a gentle warmth. The slides may be thus exposed indefinitely without danger.

Hot fixation and killing are accomplished by regulating the oven to the proper temperature and then placing the objects in a container of the desired fluid on the tray where it may be exposed for the proper length of time without danger of overheating.

For finer cytological work the oven has proved very efficient as a safe and convenient method for infiltration and imbedding. When exposure to xylol-paraffin mixtures is used, this may be accomplished on the tray as described above for hot fixation. The practice of passing the objects through several changes of paraffin may here be accomplished with all facilities conveniently placed and illuminated before the operator so that even a binocular microscope can be used when necessary, with the objects still under the proper temperature conditions. Square flat glass salt cellars are usually used as containers in this process. The button method of imbedding is facilitated if carried out on the tray where the temperature can be regulated so as to give sufficient time for the proper transfer and orientation of the objects before the paraffin is allowed to cool.

The parts of the oven are all demountable so that they may be readily removed for cleaning. Oil or gas burners or electric rheostats not entering into its construction has practically eliminated the danger of fire, and the oven may therefore be left in operation indefinitely with perfect confidence. Its light weight, compact and firm construction make it possible to transfer it with ease, and it can be kept on the individual research desk convenient for use without monopolizing this sometimes precious space.

Resumen por el autor, Bruno Oetteking.

### Patelas anómalas.

Una indicación de la inserción del músculo vasto lateral en el lado supero-lateral falta raras veces en la patela o rótula. Las inserciones en este punto producen a veces profundas escotaduras o emarginaciones como resultado de la función muscular en conexión con procesos selectivos, tales como la economía en el material formativo y las influencias del medio ambiente que ejercen su acción sobre la textura del hueso. La emarginación está a veces ocupada por patelas accesorias ("patélulas"). Las condiciones patológicas y los huesos accesorios no son los agentes causantes de la formación descrita.

Translation by José F. Nonidez  
Cornell Medical College, New York

## ANOMALOUS PATELLAE

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FOUR TEXT FIGURES AND ONE PLATE (TWO FIGURES)

Of the few anomalies met with in the human patella, that of emargination is not at all scarce. It occurs invariably on the upper outer margin of the bone, taking on a great variety of form, particularly with regard to the extent and depth of the incisure produced by the emargination. While only the extreme cases of emargination are of interest to the anthropologist, it may be well to reflect first upon the anatomical significance of the anomaly. The latter is preëminently to be understood from the mechanical or functional angle, and as such is closely involved in the articulation of the knee-joint. It has been shown that during the act of flexion the patella moves laterally under duress of the trochoginglymus character of the knee-joint, to assume an external position in complete flexion, i.e., a position in front of the lateral condyle of the femur. In this position only a narrow marginal area on the medial side of the articular surface of the patella is in touch with a similar marginal area on the lateral tibial surface of the medial condyle of the femur, forming here a distinct facet of semilunar or crescentic shape. The strain of flexion is withstood by the patellar ligament, the prolongation of, and continuous with the common tendon of *m. quadriceps femoris* and inserted in the *tuberositas tibiae*. It is this direct strain that in flexion somewhat sidetracks the patella laterally. The main agent here, as Kempson ('02, p. 420) has shown, is the tendon of *m. vastus lateralis* inserted into the upper portion of the lateral margin of the patella, in coöperation with the *tractus iliotibialis* and the *retinaculum patellae laterale* (fig. 1).

As to the variableness of insertion, there is hardly a patella found without showing at least an indication of it, quite frequently in such a case in the shape of a smooth and slightly depressed or semilunar edge of about 1 cm. or more in extension. Todd and McCally ('21), who have examined 682 skeletons, state that there

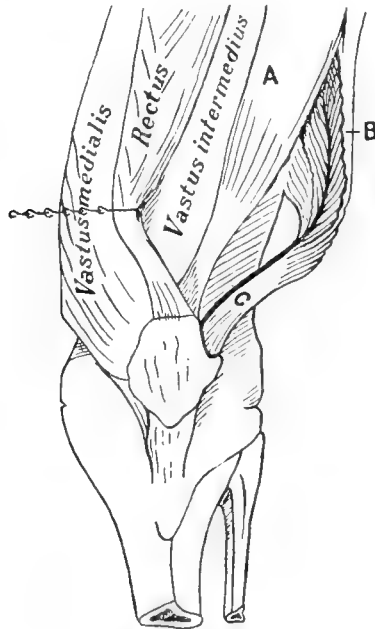


Fig. 1 The insertion of m. vastus lateralis. The upper part, *A*, of the muscle is divided from the lower part, *B*, by a longitudinal incision. The muscular fibers are seen inserting into the two surfaces of the tendon, *C*, which is inserted in the superolateral margin of the patella (modified after Kempson).

are "about three per cent of human beings characterized by more or less marked defect of the upper and outer part of the bone." This is a rather low occurrence, but of significance in view of the large material investigated. Pronounced cases of emargination with hook or spine-like projections are sometimes the result of multiple patellar formation, as pointed out by Wright ('04), who says on page 66:



The explanation which I have to offer for the emargination to the patella . . . . is that in addition to the central deposit of cartilage cells in the quadriceps extensor tendon which forms the patella, we not infrequently get an outlying deposit in the anterior fibers of the ilio-tibial band of fascia lata; that this deposit when it occurs is usually small; that it joins the central deposit, its presence being indicated by the process which gives to the patella its occasional emarginated appearance.<sup>1</sup>

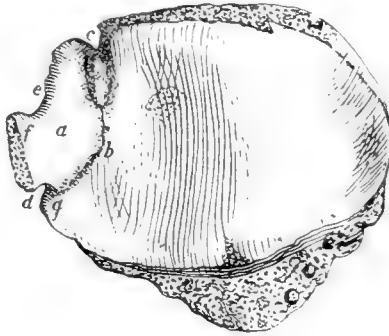


Fig. 2 Articular surface of a patella from Santa Catalina Island, California (no. 609). *a*, accessory patella ('patellula'); *b*, furrow caused by the coalescence of the patella and 'patellula'; *c* and *d*, upper and lower clefts of incomplete coalescence; *e*, insertion line of *m. vastus lateralis*, continuous with *c*; *f* and *g*, spine-like projections at lower ends of patellular and patellar notches.

That this assertion is exactly to the point in certain cases is demonstrated by a left patella in our collections from Santa Catalina Island, California (no. 609), which may be seen illustrated in figure 2. The bone shows on its upper lateral side an unevenly lozenge-shaped accessory 'patellula' (*a*), whose articular surface is set off against the articular surface of the patella proper by a distinct, but otherwise smooth and fairly deep furrow (*b*). The two elements are incompletely coalesced above and below, leaving in these two places distinct and relatively deep clefts (*c* and *d*). The anterior patellular surface, somewhat circular, is slightly bulged and rugged in continuation with the patellar sur-

<sup>1</sup> Pearson and Davis, in a comprehensive comparative study on the sesamoids of the knee-joint, assert that "in the knee-joint itself we have come across no less than ten sesamoids . . . ." ('21, p. 134), which are fittingly named by them in accord with the older nomenclature.

face, against which it is set off only by a somewhat broad and shallow depression. The anterior surface of the accessory bone is at 23 mm. almost doubly as wide as the posterior or articular one at 13 mm., while its height measures 22 mm. But the more important point here is that the 'patellula' is deeply set into a notch of the patella proportionate to the former's size. Although the theory of accessory patellae as advanced by Wright is plainly demonstrated by the case just described, it seems to be doubtful to the present writer whether the more or less spine-like projections at the lower end of the emargination unimpaired by the accessory element is due to the same origin. In none of the cases that came to his attention has he seen a demarking furrow or an indication of it between the spine and the bone proper comparable to the condition mentioned in connection with the area of coalescence between the patella and 'patellula' just described (no. 609). And again is the patellar notch, for instance, in the case to be described below, considerably deeper than simply to be caused by the spine-like projection in justification of Wright's assertion that, "the process gives to the patella its occasional emarginated appearance," which at best might only serve to emphasize the emargination. This is furthermore proved in figure 2, *c-e*, where the insertional line of the vastus lateralis muscle continues from the patella proper upon the 'patellula,' thereby transgressing the upper cleft of incomplete coalescence (*c*), and forming a spine-like projection of its own at the lower end of the patellular insertion (*f*). But the lower termination of the patellar notch that lodges the 'patellula,' also projects spine-like (*g*), and although not coalesced with the 'patellula,' the outlines of the latter and the patellar projection are continuous over the lower cleft of incomplete coalescence (*d*) to form the lateral boundary of the common articular surface. A coincidence like this in the formation of similar projections in analogous and near-by places is hardly conducive to assuming their origin from specific accessory elements. There is, however, a patella figured by Todd and McCally ('21, pl. II, no. 10), in which the spinous process at the lower end of the emargination seems to be of an accessory nature, since a distinct furrow divides the two elements, the process and the bone.

The patella in our collections (no. 697, from Chihuahua, Mexico) that called for a special investigation on account of its deep superolateral notch is rather delicate, medium sized, and of somewhat rounded or, still better, broad oval-shaped appearance. It may be seen in plate 1, as figure 5, *a* and *b*, sided with the patella just described and enumerated as figure 6, *a* and *b*. It is a left patella. Its anterior surface shows the longitudinal ridgings peculiar to this bone, and as many as twelve more or less distinct nutrient foramina. On the basis is seen the typically smooth and fairly deep horizontal groove of insertion, while the

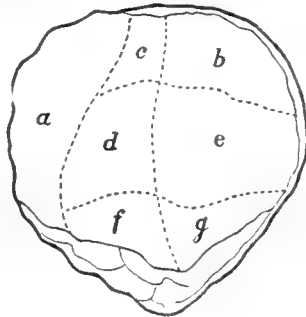


Fig. 3 Articular surface of a right patella (after Luther Holden). *a*, *b*, in contact with femur in complete flexion; *b*, *c*, in contact with femur in rectangular flexion; *d*, *e*, in contact with femur in semi-extension; *f*, *g*, in contact with femur in complete extension.

apex, bluntly pointed, appears to be smoothed down in front. On the articular side the basal margin is slightly turned inward toward the articular surface, while the rugged apex recedes from the articular surface medially to the amount of 5 mm., its lateral region being almost on a level with the articular surface. The facies articularis itself shows the typical slopings from a vertical ridge, the crista faciei articularis, toward the medial and lateral borders, to form its larger lateral and smaller medial portion. The facetation of the articular surface is also in evidence, particularly the medial vertical facet, which is markedly concave from side to side at a width of about 9 mm. and an approximate depth

of 2 mm.<sup>2</sup> The outstanding feature of this patella is the deep notch which at its lower end is pyramidally pointed, to form a spine-like projection. At its bottom the notch is considerably roughened, bearing here the two compactae and the intermediate spongiosa. The deepest place is situated at about the level of the horizontal halving line of the entire bone, or, counted from below, between the first and second thirds of the articular surface. The anteroposterior diameter between the margins of the notch amounts here to 9 mm. The distance between the two foot-points of the curve demarking the outline of the emargination measures 21 mm., while its depth comes to 5 mm. This is quite

<sup>2</sup> Besides H. Meyer ('53) it was John Goodsir who really seems to have been the first to bring conclusive evidence regarding the principal stations in the articulation of the knee-joint as expressed by certain definite marks on the cartilage of the articular surfaces, particularly of the patella. As his elaborations on the subject are of fundamental importance, but on the other hand rather difficult of access, the reprinting of part of his article may be welcome here. The present writer has supplemented it for visual information by a diagram (fig. 3) after Luther Holden (*Human Osteology*, London, 1899, 8th edition, fig. 13, p. 215).

John Goodsir ('68, pp. 224-226) writes: "The patella exhibits various interesting relations during the movements of the joint; and in addition to those observed by Meyer, others hitherto unrecorded may be pointed out. Meyer states that the under half of the patella is in contact with the femoral trochlea in extension, and the upper half in flexion; but if the bones are carefully examined, the following configuration and relation of its articular surface will be detected. Instead of two faces, a greater external, and a lesser internal, separated by a perpendicular ridge, as usually described, the surface presents, in every instance, six, frequently seven, facets, separated from one another by two perpendicular and two transverse ridges. The external perpendicular ridge is the one commonly described. The internal cuts off a small elongated perpendicular facet at the inner edge of the surface. The two transverse ridges only extend inwards to the inner perpendicular ridge, so as to separate from above, downwards, two superior, two middle, and one or two inferior facets, the external of the two latter being constant. The relations of the articular surface of the patella present four groups. In complete flexion, the internal or perpendicular facet is in contact with a remarkable crescentic facet, which bounds the oblique curvature of the inner condyle of the femur. In none of the other positions of the joint is this internal patellar facet in contact with an opposite cartilaginous surface, but is covered or sheathed by what may be denominated the internal patellar pad. At the same time, the external superior facet lies upon the fore part of the external condyle of the femur below and behind its bounding groove. These two facets are the only parts of the patella which come in contact with the proper femoral condyles. They do so only in complete flexion: and in this state all the remaining facets are in contact with the great infrapatellar pad, and the so-called mucous ligament.

considerable and becomes still more evident in comparison with the Krapina patella (Gorjanović-Kramberger, 1906, 239) whose outlines are seen sided with the Mexican one under discussion in figure 4, *a* and *b*. The absolute diameters of the two bones differ mostly in the height and thickness and may be studied from the adjoined comparative table, wherein the Spy measurements from the same publication by Gorjanović-Kramberger (p. 239) are also listed. The upper external portions of the margins of the Mexican and Krapina bones, as seen from their posterior or articular surfaces, are drawn in figure 4, *a-c*, first singly and then in superimposition, coinciding on their lines of deepest emargina-

"In the second stage of extension, the superior internal and external facets are in contact with the inferior portions, respectively, of the inner and outer halves of the femoral trochlea; the internal perpendicular facet being sheathed as before stated, and the remaining facets being in contact with the great infra-patellar pad.

"In the third stage of extension, the superior internal and external facets leave the femoral trochlea and become sheathed, and the space occupied partly by the supra-patellar, but principally by the supra-trochlear pads. The middle internal and external patellar facets now come in contact with the middle portions respectively of the inner and outer halves of the femoral trochlea; while the internal perpendicular and the two inferior facets are sheathed and padded as before.

"In the fourth or last stage of extension, the middle internal and external facets also recede from the surface of the trochlea, and along with the internal longitudinal already sheathed become applied against the fore part of the femur above its articular surface, the intervening space being stuffed by the supra-patellar, supra-trochlear, and upper pads, in the ascending cul-de-sac of the synovial membrane. In this last stage, the only portions of the patella in contact with the cartilaginous surfaces of the femur, are the inferior internal and external facets, or the latter, if one only exists. These slip somewhat abruptly upwards and inwards upon a narrow ledge or furrow, which terminates the femoral trochlea above, and forms a resting-place for the inferior facets of the patella in the complete extension of the joint."

The six or seven articular facets which Goodsir points out as typical in the European patella are naturally somewhat blurred in the dry bone. In the recent state, however, they are quite marked in the thick layer of cartilage that covers the posterior surface of the patella, the thickest in fact, in the entire skeleton, attaining a thickness of from 5 to 6 mm. over the crista verticalis. The number of articular facets does not seem to be constant, however, and their shaping is subject to the influences of function. In this respect, Lamont ('09/'10) found the medial perpendicular and particularly the lateral superior facets enlarged in the Punjabi as a result of squatting. The author appends that the same condition has been stated by Macalister as "very common in ancient Egyptian patellae."

tion. These latter measure 2 mm. and 5 mm., differing by 3 mm. in favor of the Mexican patella. It will be readily seen that the extent of the curve describing the emarginations is also greater in the latter bone where it amounts to 21 mm. against 11 mm. in the Krapina bone.

	PATELLAR MEASUREMENTS		
	Mexico	Krapina	Spy
	mm.	mm.	mm.
Width.....	44	49 46.6	51.4
Height.....	42	42.3-44.4	46.3
Thickness <sup>1</sup> .....	19	23.2-42.0	22.2-24.0
Dimensions of articular surface:			
Height.....	25		
Width.....	44		
Dimensions of emarginations:			
Length.....	21	11 <sup>2</sup>	
Width.....	9		
Depth.....	5		
Angle of articular surfaces.....	124(129°) <sup>3</sup>	109-110°	119°

<sup>1</sup> Gorjanović-Kramberger ('06, 239) lists: 'Dicke der Mitte,' but as the maximum diameter anteroposteriorly is generally found in the middle, this method should not deviate from that of the present writer's.

<sup>2</sup> Author's measurement on diagram.

<sup>3</sup> The lower figure was attained by resting the two feet of the coordinate calipers in the lateral depressions of the articular surface of the patella about coinciding with its horizontal bisector; the higher one by using the extreme margins of the articular surface, while in both cases the coordinate branch was lowered on the longitudinal crest (*crista faciei articularis*), the latter's height amounting to 9 and 10 mm. in the two cases. The proportions were then transmitted on paper and the angles measured. As regards the Spy-angle, Gorjanović-Kramberger says that it was measured 'bei der Basis,' yielding a figure then that is not directly comparable to the Mexican ones.

It is quite probable that cases of extreme emargination occur individually as a direct result of function, since man's lower limb is, with regard to the kinetics of his entire skeletal system, the most intensive functioning portion. Pathological causes, such as trauma and inflammatory processes, are fairly out of consideration here and are also dismissed as unjustifiable by Todd and

McCally ('21, p. 781), who, with regard to the causation of patellar defects, speak of various phases of an anomaly of patellar ossification. There is, however, another moment that may not be overlooked, namely, nature's apparent tendency toward economy in formative material. In the higher types this gives rise to the formation of muscle marks, such as tuberosities, ridges, and the like, which in the inferior types and the anthropoid apes are frequently seen only sparsely developed, a greater amount of osseous mass taking the place here of more differentiated conditions. Still another consideration would be that with regard to the bone whose less resistive qualities as a result of the influences

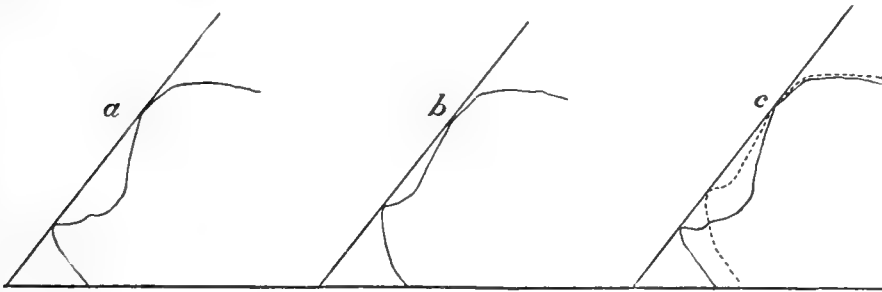


Fig. 4 Comparison of outlines showing emarginations of: *a*, the Mexican; *b*, the Krapina patella; *c*, superimposition of *a* and *b*. Natural size.

of more refined environmental conditions might, in conjunction with the above-named economy in formative material, be responsible for the specific relief known as muscle marks. Under that consideration a muscular insertion might eat itself deeply into certain bones like the patella to form the deep notch found there at times.

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## PLATE 1

## EXPLANATION OF FIGURES

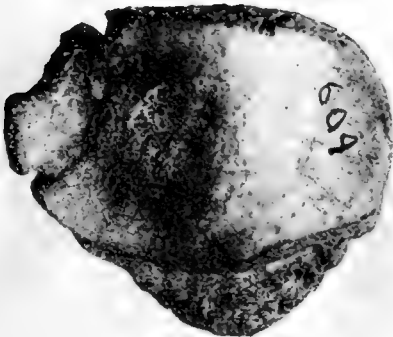
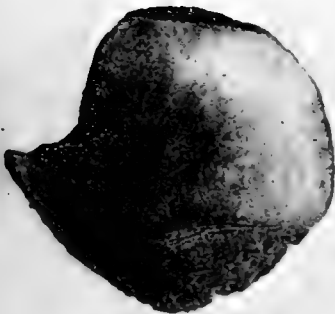
Anomalous patellae, figures 5 and 6, *a* and *b*, represent the articular and anterior surfaces of two anomalous patellae from Chihuahua, Mexico, and Santa Catalina Id., California, the former marked by a deep insertional notch, the latter by an accessory bone ("patellula") filling up to the greater part a well marked notch.



a

5

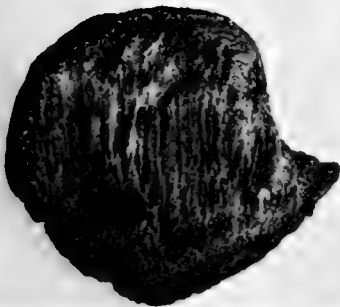
b



a

6

b



080

## BOOKS RECEIVED

TRAITÉ D'EMBRYOLOGIE DES VERTÉBRÉS, par A. Brachet, Professeur à l'Université de Bruxelles: xv + 602 pp., 567 figures; Paris, Masson et Cie., 1921.—At present, the standard French text-book of embryology. The author states that its plan is wholly different from that of corresponding texts in Germany, England, and the United States; and its purpose is in fact quite unlike that of the Minot and Prentiss-Arey laboratory manuals, and of Bryce's or Keith's English works. It perhaps most resembles Hertwig's Lehrbuch. The general part, devoted to the first phases of ontogenetic evolution of the vertebrates and to the establishment of the great fundamental laws on which they depend, has, according to the author's preface, been developed in a manner befitting the importance of the subject, but chapters which are scarcely more than introductory to the study of histology or comparative anatomy have been somewhat summarily written. "A career long devoted to comparative embryology" has enabled Professor Brachet "to observe for himself many things and to arrive at personal opinions on many questions," the results of which appear throughout the book. The numerous figures have been well reproduced, and the paper and printing are good, despite the difficulties of "the present hour."

CAPITA ZOOLOGICA: VERHANDELINGEN OP SYSTEMATISCH-ZOOLOGISCH GEBIED, onder redactie van Prof. Dr. E. D. Van Oort, Directeur van 's Rijks Museum van Natuurlijke Historie Te Leiden. Deel I, Aflevering 1, Dr. J. G. De Man—Nouvelles Recherches Sur Les Nematodes Libres Terricoles; (62 pages, 10 x 13, 14 plates). Deel I, Aflevering 2, Dr. G. Stiasny—Studien über Rhizostomeen, (180 pages, 5 plates): 's Gravenhage, Martinus Nijhoff, 1921.

A new Dutch zoölogical periodical is being published (The Hague) in the English, French and German languages. It includes transactions on systematic-zoölogical subjects, each number forming a complete work. The issues are sold separately. A volume contains about 500 pages including illustrations. The above described two numbers were issued in 1921.

LABORATORY APPARATUS AND REAGENTS Selected for Laboratories of Chemistry, Metallurgy and Biology in their application to Education, The Industries, Medicine and the Public Service, 8 x 11½, 620 pages, profusely illustrated; with additional matter consisting of Code Index and Condensed Price List, 72 pages; Reagents for analysis, Bacteriology, Microscopy and Metallurgy, 104 pages and a 20 page Index; Philadelphia, Arthur H. Thomas Company, 1921.

This new edition of the Catalogue in stiff cloth cover not only has ample and exact descriptive text, with full reference to scientific publications involved and methods of use (where such are likely to be of interest) and accurate illustrations as in previous editions, but also contains several new features; actual prices (other than daily market changes), contents of original packages, Code Index giving code word for each size of every item listed, and Reagent listing with code word for every size of original package, explanation of export terms, preparation of documents and method of packing for export. The printing, papers used, and binding are excellent.

*(Continued on page 322)*

Resumen por el autor, Paul H. Stevenson.

Sobre un músculo digástrico anómalo en el muslo de un chino.

En el presente trabajo se da a conocer una observación sobre la presencia de dos vientres musculares supernumerarios unidos con los músculos biceps y piriforme del fémur, respectivamente, y contínuos entre sí mediante un tendón intermediario asociado con la fascia que se extiende sobre el gran trocánter. Las relaciones de los músculos con las estructuras vecinas, así como las relaciones que presentan entre sí, indican la existencia de una sola anomalía del desarrollo, la cual produce un músculo de forma digástrica, en vez de dos músculos aberrantes separados que se reúnen después de su llegada a un punto común anormal. El autor revisa las relaciones embriológicas tempranas de la región, y aquellos estados de la diferenciación de los esbozos musculares tempranos que tienen importancia en la anomalía descrita son objeto de discusión. En adición a la anomalía objeto del presente trabajo el autor enumera otras siete anomalías observadas en los músculos apendiculares y en los nervios del mismo sujeto.

Translation by José F. Nonidez  
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## ON AN ANOMALOUS DIGASTRIC MUSCLE IN THE THIGH OF A CHINESE

PAUL H. STEVENSON

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### ONE FIGURE

Failure to find in the literature any citation of the following variations in the musculature of the lower extremity suggests the value of placing the present case on record. From the standpoint of ontogeny, the relations found present a picture of such unusual interest that a brief discussion of the developmental factors concerned is also included in this report.

### DESCRIPTION

The anomalies to be described represent the most important of several observed during the dissection of a male Chinese, age 39. An enumeration of the other abnormalities observed in this same body is appended at the end of this report.

Lying beneath the long head of the biceps femoris and roughly bisecting the angle between that and the short head, there was discovered a third distinct, though smaller head to this muscle. The fibers of this supernumerary muscle slip, which measured approximately 15 cm. in length and from 3 to 5 cm. in diameter, converged proximally into a small tendon which passed out of sight in the earlier stages of the dissection between the peroneal and tibial divisions of the sciatic nerve (fig. 1). At the same time there was observed a smaller cord-like tendon emerging from beneath the lower border of the gluteus maximus above and passing distomedially toward the deeper structures of this region of the posterior thigh. Although the course of this latter tendon formed a distinct angle with that of the tendon arising immediately from the supernumerary head of the biceps, yet

manipulation and subsequent dissection proved their continuity one with the other. In brief, the tendon of this anomalous muscle, continuing proximally from the distodorsal position of

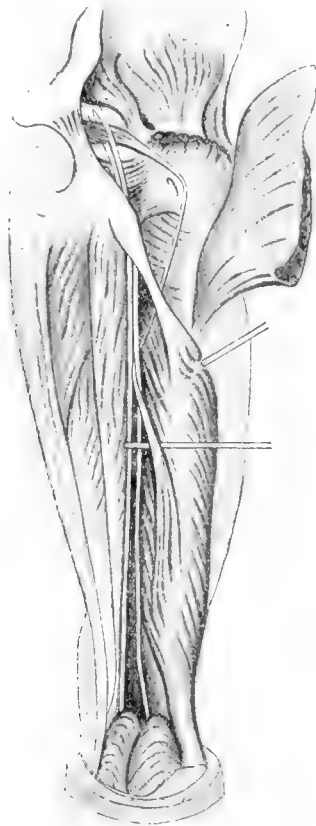


Fig. 1 A semidiagrammatic drawing of the posterior musculature of the thigh in which the anomalous digastric muscle appeared, showing the relations of the two muscle bellies to the piriformis and the biceps, respectively, as well as the relation of the intervening tendon to the peroneal division of the sciatic nerve and to the greater trochanter.

the muscle below, passed dorsoventrally around the medial side of the peroneal nerve and, after becoming loosely associated with the fibrous tissue investing the divided sciatic nerve, changed its course from a slightly medial to a slightly lateral direction (fig. 1).

Upon reflecting the gluteus maximus muscle and tracing the proximal portion of this anomalous tendon to its origin, it was observed to be associated with the capsule of the bursa lying between the gluteus maximus and the greater trochanter (*b. trochanterica m. glutei maximi*).

Careful dissection of the origin of the above tendon and examination of the relation of its fibers to those of the superficial layer of the bursa capsule revealed the fact that the tendon did not end there. Although sending out several diverging strands of fibers which became intimately incorporated in the capsular connective tissue, the tendon itself remained more or less distinct from the structure of the capsule and continued across it as a conspicuous though slightly flattened band (fig. 1).

The subsequent course of the tendon after leaving the surface of the bursa capsule at its proximomedial border was directed strongly medialward. The tendon now ran roughly parallel to the lower border of the piriformis muscle, being separated from the latter by a well-defined space of from 1.5 to 2 cm. above as well as by an interval corresponding to the thickness of the dorsal margin of the greater trochanter ventrally. In the case of the left side this tendon remained a fibrous cord throughout the rest of its course. On the right side, however, the tendon served as the attachment of a small muscle slip, from 1 to 3 cm. wide and approximately 10 cm. long, which passed ventral to the peroneal nerve and then between this and the tibial nerve as these emerged as separate nerve trunks from the pelvis, thereby duplicating in respect to the peroneal nerve the relations of the distal portion of the tendon below the capsule (*v. supra*). This proximal muscle slip continued medially to its origin on the anterior aspect of the third and fourth sacral vertebrae and from the dorsolateral surface of the sacrospinous ligament.

These two aberrant muscle slips were innervated by a twig from the tibial and a small branch of the anterior division of the second sacral nerve, respectively.

## DISCUSSION

The embryological interpretation of the anomalous condition just described is not at once obvious. A brief discussion of some of the more relevant factors involved may therefore be considered not out of place.

In the first place, it may readily be suggested that we are dealing simply with two aberrant muscle slips—arising from two well-defined muscle entities, viz., the biceps femoris and piriformis muscles, known in each case not infrequently to give rise to supernumerary slips—whose tendons have secondarily become associated with each other in the connective tissue overlying the greater trochanter as described above. Testut,<sup>9</sup> Schaeffer,<sup>8</sup> Frohse and Fränkel,<sup>5</sup> LeDouble,<sup>7</sup> and others have not only reported original cases, but have compiled long lists of supernumerary heads and aberrant slips observed in connection with the biceps femoris. To them the reader is referred for a detailed enumeration of these cases. When these reports are closely analyzed, however, and due allowance made for differences in terminology and interpretations, it is found that the range in variation in the origin and insertion of such slips is not wide. Testut (l.c., p. 993) summarizes the eight or nine distinctive types reported in the literature up to 1911 as follows:

“Le muscle biceps peut être renforcé par des faisceaux surnuméraires, provenant suivant les cas: 1° du condyle externe (Gruber); 2° de la partie supérieure de la ligne âpre (Meckel); 3° de la ligne rugueuse qui réunit le grand trochanter à la ligne âpre (Giacomini); 4° du fascia lata (Hénle); 5° du vaste externe; 6° du grand adducteur.—J’ai observé chez un nègre (Dissection d’un Boschiman, in *Arch. du Muséum*, 1885), un faisceau surnuméraire, de forme triangulaire, qui partait du coccyx pour aller grossir la masse commune du biceps et du demi-tendineux.—Turner a signalé (*Jour. of Anat. and Phys.*, 1872), sous le nom de *tensor fasciae poplitealis*, un petit faisceau musculaire qui se rendait du biceps à l’aponévrose poplitée.—Des faisceaux plus singuliers encore, se rendant du biceps au tendon d’Achille et au calcaneum, ont été observés par Kelch (*Beitr. z. path. Anat.*, 1813), par Gruber (*Beobacht. aus d. menschl. u. vergl. Anatomie*, Heft. II, Berlin, 1879) et par Halliburton (*Jour. of Anat. and Phys.*, 1881).<sup>9</sup>



Careful reading of the original of as many of the above references as were available, not only for the particular variation reported in each, but also for the review of such supernumerary slips of the biceps femoris in general that is usually contained in these writings, failed to reveal, however, any record of such an aberrant head taking origin from the fascia (either entering into the formation of the bursa capsule or otherwise) over the greater trochanter. Of the possible exceptions to this, the cases of the Giacomini and of Henle quoted by Testut (*v. supra*) seem to refer in the first instance to a definite bony origin from a line on the femur between the greater trochanter and the *linea aspera*, evidently a slip from the short head of the biceps having an independent origin slightly proximal to the usual origin of that head on the *linea aspera*, and in the second instance to a more superficial fascial slip from the fascia lata to the long head of the muscle. LeDouble (*l.c.*, p. 281) also mentions a supernumerary fascial attachment "*de la face profonde du grand fessier, à quelques centimètres de ses insertions iliaques*" which, although associated with the fascia of the deep surface of the *glutaeus maximus*, seems to be distinct from the instance herewith reported.

The second muscle slip with which we are concerned in the present view of the anomaly is the proximal fleshy termination of this aberrant tendon. Continuing medially and proximally from the trochanter and passing ventral to the peroneal nerve and then dorsally between it and the tibial nerve trunk to its final origin from the sacrum, this muscle slip would probably be considered in such a scheme simply as a divided portion of the *piriformis*. Both in point of origin and in its relation to the two divisions of the sciatic nerve this muscle slip qualifies well for such a classification, and the condition, if indeed this is the condition with which we are dealing, is found frequently enough to warrant the statement that it occurs normally in about 20 per cent of all cases.<sup>4</sup> Such divisions affect only the proximal portion of the muscle, however, and almost invariably reunite with the main *piriformis* muscle tendon to become inserted with it upon the anterior and inner portion of the upper margin of the

greater trochanter. A complete separation of such slips from the usual fibers of the piriformis at the point of their insertion, on the other hand, and a subsequent migration of their insertion from the inner aspect of the trochanter downward and outward to a position on the lateral side of the trochanter, as apparently has occurred in the present case, has never been noted in the literature as far as the writer has been able to ascertain. Upon the occurrence of a continuation of such an aberrant tendon from the piriformis with an equally aberrant tendon which has reached the same point from the biceps femoris below, the literature is similarly silent. The difficulties in the way of supplying this latter phenomenon with a logical developmental background are obvious.

The second view of the anomalous condition as observed in this case suggests that we are dealing with a single developmental anomaly resulting in a compound muscle of digastric form. The early work of Bardeen and Lewis<sup>1</sup> and the later studies of the former<sup>3</sup> leave little doubt that the anlagen of such muscle aberrancies as those with which we are here dealing become differentiated as such, either during or very soon after the period of general differentiation of muscle groups within the primitive myogenous zone of the limb. Unfortunately, none of the excellent figures in the articles just referred to were drawn from an angle showing the respective anlagen of both the biceps femoris and the piriformis at the same time. A careful study of these figures of actual reconstructions of the developing nerves and muscle of the lower limbs in very early human embryos, however, gives good evidence of two facts having a direct bearing on the possible formation of the anomaly in question.

In the first place, in the still undifferentiated myogenous zone of the limb bud up to the 7- or 10-mm. stage, those concentrating areas of cells which are destined to differentiate later as the specific anlagen of the biceps and piriformis muscles lie very close together. The only structure intervening in the following stages of progressive differentiation in this region is the outgrowing sciatic nerve trunk. This means that in those cases where the sciatic nerve, either as a whole or in part, pierces the piriformis

muscle a portion of the anlage of this muscle is still less definitely separated from that of the biceps which lies immediately adjacent. Such a split-off portion of the piriformis anlage, as mentioned above, almost invariably again associates itself with the main body of the muscle to become incorporated in the common tendon of insertion, after transmitting a portion or all of the sciatic nerve through the resulting proximal hiatus. Failure to reunite with its parent anlage, however, would present the alternative possibilities either of an independent existence or of an association with a neighboring muscle anlage. Both of these fates of aberrant slips from the piriformis are apparently rare in man, but that such an association with the adjacent biceps sometimes occurs in the case of lower forms may account for the original opinion of Gegenbaur<sup>6</sup> (since corrected by later observers), that the piriformis was derived from the caudofemoral muscle of urodeles—a muscle represented in many mammals by a caudofemoralis extending from the caudal vertebrae to the lateral side of the distal half of the femur.<sup>2</sup> The writer believes that the present case represents an example of such an association between a separated portion of the piriformis anlage and that of the biceps femoris in man. The picture in this case is somewhat complicated by an intervening attachment over the greater trochanter, which will be discussed below, but it is altogether probable that the case of a sacral attachment of the biceps attributed to Macalister (original reference not given) by LeDouble (l.c., p. 281) represents an uncomplicated case of such an embryonic association between these respective muscle anlages.\*

The second relevant fact derived from a study of the reconstructions of early human embryos referred to above is the relations of the developing skeletal elements in the primitive scleroblastema with reference to the two muscle anlages dis-

\* The sacral attachment of the biceps here under discussion must not be confused with the frequent fasciculi from the coccyx, sacrum, or sacrotuberous ligament to the long head of the biceps, giving rise to the opinion of some investigators that the long head of the biceps represents a muscle primitively arising from the sacrum or coccyx and that the sacrotuberous ligament represents a transformation of that part of the muscle originally extending between the sacrum and its present origin from the ischial tuberosity.

cussed in the preceding paragraph. These are more clearly emphasized in the further studies of Bardeen devoted entirely to the development of the human skeleton. Up until a stage of development represented by a 14-mm. embryo (embryo CXLIV, pl. III, figs. 5 and 6, Bardeen<sup>2</sup>), or considerably after the beginning of the muscle-anlage differentiation, the developing femur has maintained its primitive position in relation to the pelvis. At this time the shaft of the femur lies approximately at right angles to the body axis and extends directly into the hip-joint. Here there is no neck, merely a simple rounded head, distal and dorsal to which a slight projection marks the beginning of the greater trochanter. Thus the primitive anlage of the trochanter lies not only in direct line, but also practically in the same plane with the two muscle anlages discussed above. The possibility of a secondary association between the aberrant muscle-slip anlage herewith implied and the developing trochanter lying immediately ventral to it would be enhanced in the case in question by the tendency naturally inherent in any divided portion of the piriformis to seek an attachment on the trochanter.

By the time the development of the embryo has progressed to a stage represented by one of 20 mm. (embryo XXII, pl. IV, figs. 9 and 10, Bardeen<sup>2</sup>), the trochanter is not only clearly differentiated as such, but has also rotated lateralward from its more primitive position. This lateral rotation and subsequent shifting of the trochanteric region incident upon the progressive development of the future conformation of the proximal end of the femur, dragging with it the blastemal attachments already formed—which in the case in question would include that of the aberrant muscle anlage bridging the gap from the piriformis above to the biceps below—would account for the picture presented in adult life (fig. 1).

The writer is led to suggest this second interpretation, therefore, as the true explanation of the appearance of this muscle of digastric form in the musculature of the posterior region of the thigh of this Chinese subject. Rather than with two aberrant muscle slips arising independently from distinct and widely separated muscle entities, effecting a unique union after pursuing

courses equally unusual, it seems more probable that we are dealing with the development of a single muscle anlage, the central and later tendinous portion of which early became associated with an adjacent skeletal element whose subsequent rotation from its primitive position obscures somewhat the fact of the essential continuity of the widely separate muscle bellies.

In addition to the anomaly reported above, the following were also observed in this same subject: 1) excessive size of the peroneus tertius muscle in both legs; 2) absence of the tendon of the flexor digitorum brevis usually passing to the little toe; 3) supernumerary slip arising from the tendon of the flexor digitorum longus beneath the lacinate ligament and dividing into two tendons inserting into the lateral side of the flexor digitorum brevis and the second phalanx of the fifth toe, respectively; 4) total absence of the plantaris muscle on the left side and its reduction to a fibrous cord on the right; 5) the anterior trunk of the seventh cervical nerve joins that of the eighth to form the medial cord of the left brachial plexus, while on the right side the anterior trunk of this same nerve divides into two divisions of approximately equal size joining the lateral and medial cords of the plexus, respectively; 6) supernumerary heads of both biceps brachii arising in each case from the middle third of the medial side of the humerus; 7) a small aberrant muscle slip in the right forearm, having its origin in common with the flexor digitorum sublimis and becoming tendinous soon after its origin and passing down the arm and beneath the transverse carpal ligament as a slender tendinous cord which inserted in the palm into the tendon of the flexor digitorum profundus passing to the third finger.

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Resumen por el autor, C. Judson Herrick.

Algunos factores en el desarrollo del sistema nervioso  
de los anfibios.

El autor considera tres aspectos del problema del origen y desarrollo del sistema nervioso, a saber: 1) Los factores hereditarios de origen muy remoto, tales como el metamerismo primitivo; 2) Los factores hereditarios de origen mas reciente, de significación adaptativa evidente, tales como los patrones de reflejos; 3) El efecto inmediato de la función activa sobre el progreso del desarrollo individual.

En la investigación de los factores segundo y tercero es necesario primeramente descubrir qué representan estas adaptaciones funcionales y como están estructuralmente representadas en el cerebro. La localización anatómica de los sistemas funcionales específicos del cerebro del *Amblystoma* adulto ha sido determinada y este patrón anatómico puede seguirse hacia atrás en la serie embriológica hasta los estados tempranos. La correlación de la estructura de estos estados del desarrollo con los tipos de reacción de especies individuales es al presente objeto de investigaciones. Después se determinen de este modo estos factores funcionales adaptativos en la morfogénesis, el análisis ulterior del proceso del desarrollo mediante métodos experimentales y de otra clase puede llevarse a cabo con mayor facilidad. En el presente trabajo se ha incluido un resumen de los trabajos más recientes sobre el sistema nervioso de *Amblystoma*, así como indicaciones para la investigación cooperativa.

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## SOME FACTORS IN THE DEVELOPMENT OF THE AMPHIBIAN NERVOUS SYSTEM

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Three factors, amongst others, may be recognized in the embryologic development of the brain:

1. Very ancient palingenetic hereditary influences, such as primitive metamerism. These doubtless originally had functional significance, but this is now largely lost and these vestigial features are in most cases masked or suppressed early in developmental history.

2. Obviously adaptive features which are firmly hereditary but of relatively recent (cenogenetic) origin, e.g., reflex patterns and their neuromotor apparatus.

3. The immediate effect of active function upon the progress of individual development—facilitation by use, trophic response of the tissue to excitation, direct action of physiologic gradients of excitation and conduction upon direction and rate of growth (Child, '21), the bioelectric phenomena of excitation and differentiation and their directive influence upon contiguous differentiating neuroblasts (Lillie, '19; Kappers, '17, '21; Bok, '15, '17; Ingvar, '20), hormone action, and probably many others. Some of these are critically discussed by Child ('21).

Students of the embryology of the brain have been inclined to stress the first of these factors, to touch lightly upon the second, and to dismiss the third as irrelevant to their studies. Recent years, however, have seen a notable change brought about largely

<sup>1</sup> This summary has been written up in collaboration with a number of active students of amphibian development, and it seems to us that its publication at this time would serve a useful purpose in promoting more effective integration of efforts now somewhat dispersed among scattered workers by directing attention to certain features of these researches which look toward problems of more general significance.

by improvement in the technical methods of experimental analysis of the problem, and the demand now is for coördinated attack upon the problems of development on all these frontiers.

To learn the rôle of functional adaptations of the second and third types, obviously it is first necessary to determine what these adaptations are. As applied to the development of the central nervous system, this implies a more complete knowledge of the functional localization and functional interrelationships of parts than we possess at present for any animal except man—and even here our ignorance is more extensive than our knowledge. The inconceivable complexity of the human brain is a serious obstacle to such an analysis and, moreover, the mammalian nervous system does not readily submit itself to the methods of experimental embryology.

The amphibian nervous system is free from the two objections just mentioned and in addition it has the further advantage that it begins to function in response to external stimulation at a surprisingly early stage of development. Complex adaptive reflex mechanisms which in the human nervous system attain full structural maturity before they are called upon to act at all can in a series of young amphibian tadpoles be observed in function at progressively advancing stages from the simplest beginnings to very complicated definitive forms, and the structures which are added from stage to stage of the increasingly complex reflex pattern can be identified and correlated with the specific parts which they have to play in the total physiologic complex.

Intensive studies of the development of *Amblystoma*, both observational and experimental, have been in process for a long time. Coghill has observed the earliest reactions of urodele larvae to external stimuli and determined the sequence of progressively more complex behavior patterns. These observations were first made on *Diemyctylus* ('09) and have since been extended to *Amblystoma*. The earliest reactions of these larvae to contacts on the head and trunk are of three types, but all immediately or within a few hours come to a form in which for from 48 to 96 hours the head is regularly moved away from the side touched, an avoiding reaction. This 'early flexure' stage is followed successively by

a series of stages leading up to the 'early swimming' stage. Later experiments on *Amblystoma* have shown that the 'early swimming stage' is followed by 'non-responsive' stages (to tactile stimuli around the mouth), and these by 'feeding' stages.

Coghill and his pupils have in progress an extensive study of the structural changes correlated with this sequence of clearly defined functional stages, some parts of which have been published ('13, '14, '16). The sequence of maturation of the elements of the neuromotor mechanism is found to present a very instructive parallelism with the demonstrated functional sequence and important contributions have been made to the question of the nature of the most primitive reflex mechanisms (cf. Herrick and Coghill, '15).

Mention should be made in this connection of the valuable studies made by Paton ('07) on various species of vertebrates which, though not parallel with Coghill's, were similarly directed, and of the still unpublished work of Dr. H. C. Tracy on the toadfish, *Opsanus*. Both of these include the study of spontaneous movements and also reactions to external stimulation. Tracy has already carried his study of the sequence of the development of the nervous system in correlation with observed behavior to an advanced position. While the details of the development of the reflex patterns of the toadfish are naturally very different from those of amphibians, yet there are some general principles of development common to the two which are very instructive, and the differences between the two species are not less so as illustrative of adaptive changes correlated with the long period during which the toadfish larva is firmly attached in a protected situation.

The present writer has attacked the problem from the opposite side and is engaged upon histological studies of the adult and late larval nervous systems of *Amblystoma* and other urodeles, taking as the point of departure Coghill's earlier ('02) analysis of the functional components of the peripheral nerves. The determination of the exact pathways between the peripheral end-organs and their cerebral centers has permitted an accurate mapping of the primary reflex centers of the brain—tactile, gustatory,

visual, olfactory, etc., and the various motor nuclei. Anatomical continuity between these end-organs and their respective cerebral centers gives a true indication of the functions of the latter. This has been adequately controlled physiologically. By an application of the same principle anatomical analysis of the complex central fiber tracts which connect these primary centers with each other and which connect them with their respective higher correlation centers yields definite information regarding the functions of the higher centers.

The ultimate court of appeal in searching for the precise localization of function in the nervous system is, of course, direct experimentation; but there are many regions of the central nervous system which are as yet inaccessible to such experimental studies, and the anatomical methods just mentioned under proper control are here very serviceable.

The brain of adult *Amblystoma* is very simply organized, with relatively little departure from the form of the early embryonic neural tube, except for the great flexures and the local thickenings of the wall produced by the various correlation centers to which reference has just been made. To these there is added in the forebrain a considerable outpouching or evagination of the entire wall on each side, thus producing two hollow cerebral hemispheres whose pattern is in fundamentals similar to that of the hemispheres of the human brain (cf. Herrick, '21).

Beginning with an analysis of the primary reflex centers of the medulla oblongata and their secondary connections in *Amblystoma* of mid-larval stages (Herrick, '14a), the higher connections of these secondary tracts and their relations to optic and olfactory centers have been determined. These studies are far advanced and, through the united labors of several neurohistologists, we have now a fairly complete knowledge of the functional pattern of the brains of *Amblystoma* and other urodeles. Among the more important contributions to this subject, mention may be made of papers by Kingsbury ('95), Herrick ('10, '14, '17, '21a), Röthig ('11, '11a, '12), Bindewald ('14), Larsell ('20). The writer also has a considerable mass of observations as yet unpublished.

Notwithstanding the simplicity of external form of these urodele brains, the knowledge of the functional localization derived from studies of the fiber tracts permits the assignment of tolerably precise functional meanings to numerous external landmarks. In short, the reflex patterns as functional entities can be correlated with a mosaic of structural pattern, part of which is externally evident. The neurons of the correlation centers occupy more space than do the related fiber tracts. Accordingly, the location of these centers is indicated by the sculpturing of the external and ventricular surfaces of the brain.

The details of the ventricular structure of the brain of adult *Amblystoma* have not yet been published (for *Necturus*, see Herrick, '17), but these have been carefully charted and their functional connections determined. It is, therefore, possible to define each ventricular eminence and external landmark in terms of functional correlations there effected and to compare these with corresponding regions of the human brain so far as the latter are represented in the *Amphibia*.

The morphological analysis of the amphibian forebrain published by the writer in 1910 was based largely upon adult material. How far this will stand the test of further embryological and experimental control remains to be determined. It is evident that these conclusions, in so far as they may prove valid, are expressions chiefly, perhaps wholly, of the second and third types of process mentioned at the beginning of this sketch. As was recognized at that time, they are not derived from primitive metamerism, the longitudinal columns of His, or other palingnetic features of that category; but rather they represent later structural modifications superposed upon those ancient patterns.

The next step is to read this mosaic pattern backward into successively younger stages of the development of the brain, identifying each region by its superficial markings or internal fibrous connections or by both of these criteria. This is readily accomplished for each functionally defined area as far backward in the series as its functional fiber-tract connections are demonstrable.

It is found, as the series is followed forward from younger to older stages, that the invasion of a given undifferentiated area by a definite fiber tract arising in a remote part and conducting some specific functional type of nervous impulses is accompanied by a pulse of local differentiation. As the various fiber tracts of the forebrain successively mature and functional connections are made with different regions, there is an immediate structural response in the form of more rapid local proliferation of neuroblasts in the areas newly invaded by the growing fibers. This results first in a thickening of the wall in the differentiating areas on the ventricular side, followed in many cases either by corresponding thickening on the lateral surface or by a local bowing out or buckling of the entire wall producing an evagination.

This localized acceleration of growth can in some cases be correlated with a definite increment, on the side of behavior of the living tadpole, in the complexity of the reflex pattern. The pulse of local differentiation is, therefore, probably very largely of the third physiological type to which reference was made in the beginning, viz., a direct reaction of the embryonic tissue in question to excitation entering from without its own substance, or a true functional response. This excitation is not necessarily a functional response to the stimulation of an end-organ of adult type, but it may be a change in metabolic rate or bioelectric state produced locally by the ingrowth of new axons into indifferent embryonic tissue (cf. the experiments of Ingvar, '20, and the transplantation experiments of Burr, '20).

But it is possible to read this mosaic pattern of regions which in the adult are obviously functionally defined back to still younger stages in some cases, that is, these regions present recognizable morphological criteria at stages antecedent to the appearance of functional fiber-tract connections. Regions which in the adult are of high functional value, in prefunctional stages may exhibit an acceleration of growth resulting in various local thickenings and dilatations of the neural tube and the great flexures. This would seem to imply hereditary factors of the type characterized by Cope ('87, p. 126) as heterochronous accelerated development, to wit, the appearance of adaptive struc-

tures at a date earlier in the ontogeny than would be consonant with an exact recapitulation of the phylogeny. How far this type of differentiation is due to the actual inheritance of morphological pattern such that particular parts of the neural tube tend to unfold in a predetermined way through the action of resident forces, and how far this sequence of changes in a particular region is due to the present influence of morphogenetic factors acting upon it from its intraorganic environment, such as Child's gradients, hormones, etc., is an approachable problem.

An important first step would seem to be the determination of the exact functional pattern which in the final stage—the adult brain—is reflected in the structural mosaic of the definitive neural tube. A second step carries this mosaic pattern back to the earlier stages of the ontogeny as far as the functional factors of the adult type can be shown to be operative, that is, to the first appearance of the several fiber-tract connections of known functional significance.

After the morphogenetic influence of these factors is determined, the residual factors must be further analyzed, and here a variety of methods are available. Some of these methods are purely observational, some are experimental.

The embryology of the Amphibia has been much studied. The only detailed account of the early development of the nervous system of *Amblystoma* is that of Griggs ('10), though there are several good accounts of the formation of the neural tube and the earlier phases of its differentiation of other urodeles. No description of the later stages of the brain of *Amblystoma* has been published.

Landacre is investigating the cranial ganglia of urodeles and has published ('21) an important paper giving the history of the neural crest. He describes extensive contributions of cells from this and other ectodermal sources to mesodermic structures (formation of mesectoderm), and follows the history of some of these cells through to their ultimate differentiation into head cartilages. This commingling of cells of the same origin as those of the brain with the cells of entodermal mesenchyme in the formation of non-nervous organs of the head may play some rôle as yet

undetermined in the equilibrated systems of growth. The relations of the definitive cranial ganglia to the neural crest and of both of these to the neural tube on one hand, and to the general ectoderm, on the other hand, have been investigated by Landacre in a number of vertebrates, and further studies of these questions and of the formation of mesectoderm in *Amphibia* are in process. Coghill has published ('16) graphic reconstructions of the functional composition of the cranial ganglia and related sensory centers of the medulla oblongata of *Amblystoma* in four of his functionally defined stages.

The further prosecution of these embryological studies will contribute much of value in the analysis of the morphogenetic factors operative in the development of the nervous system of *Amblystoma*. We wish to know what was the primitive segmentation of the urodele head and the fate of these segments in the later development of the nervous system, what are the sources from which the various parts of the central and peripheral nervous system have drawn their building material (these apparently differ in closely allied species); what (if any) are the inherited potencies of these various kinds of material; to what extent and in what ways do excitations acting directly upon them shape the course of their development; and the evidence (if any) of the presence of inherited patterns of adaptive (cenogenetic) type in this development.

Evidently observational methods alone are incompetent to answer all of these questions, but observation must furnish the sound basis upon which crucial experimental tests can be devised. And it is indispensable that the observational studies and the experimental program be kept in the closest possible articulation. Coghill, for instance, is engaged upon a correlation of the growth rhythms of the central nervous system (as measured by the number and distribution of mitotic figures at successive stages of known physiological type) with those of various non-nervous organ systems. This may shed light upon the nature of the physiological equilibrium in the organism as a whole during the march of these events, and it would not be at all surprising if these observations would correlate in a fruitful way with studies on the morphogenetic influence of the ductless glands.



Experimental investigation of the interrelationships of the endocrine organs during development has already brought to light some surprising facts. As illustrations of these, mention may be made of the studies upon the Amphibian hypophysis of Allen ('16, '20) and P. E. Smith ('20). These exhibit the influence of an organ upon remote parts of the body through the agency of transported materials.

It has long been supposed that contiguous parts may exert a chemotactic or other influence upon the development of the nervous system, and the recent studies of the bioelectric phenomena of metabolism, and especially of excitation and conduction, to which reference has already been made, have suggested a possible mechanism for this type of reaction of the tissues. The investigation in the Amphibia of the physiological gradients to which Child has devoted so much attention has been begun by Bellamy ('19). He has studied the effects of various toxic and depressing agents upon the gradients in physiological activity along the axes of the embryo and the rôle of the normal physiological gradients as morphogenetic factors. Publication of further results of these experiments is promised, and it may confidently be expected that additional data of far-reaching importance in the analysis of the functional components in the complex history of the development of the nervous system will be brought out.

From the dawn of experimental embryology until now the Amphibia have presented most favorable materials for experiments in this field. Limitations of space here forbid reference to even the most important of these investigations, but it is generally recognized that this avenue offers a very direct approach to the problems centering about the relations of developmental factors intrinsic to an organ or tissue and those which are the expressions of extrinsic agents. In this country *Amblystoma* is a more satisfactory subject for many of these lines of inquiry than any other type. The genus is widely distributed, the eggs are easily collected and reared, and the larvae are hardy and favorable for operative work. The activities of Harrison's laboratories in recent years have given evidence of the peculiar fitness of this material for many lines of embryological study.

Burr in three recent studies ('16, '16 a, '20) has opened a fruitful line of investigation by a direct frontal attack upon the question of the relation between intrinsic and extrinsic factors in the morphogenesis of the cerebral hemispheres of *Amblystoma*. By an ingeniously planned series of experiments he has shown that the hemisphere develops under the influence of excitations entering at opposite ends, from the olfactory organ and the thalamus, respectively. The destruction of the nasal organ in prefunctional stages results in the failure of the rostral end of the hemisphere to complete its differentiation. The effect of cutting off all nervous impulses coming into the hemisphere from the thalamus was determined by transplanting the hemisphere, before the ingrowth of such fibers, into the skin of the side of the body. The olfactory placode was transplanted with the hemisphere and made connection with the brain in the normal way. The hemisphere developed to nearly normal size, but the parts normally directly connected with the diencephalon were retarded in development. No part was totally atrophied, in conformity with the well-known fact that practically all parts of this hemisphere receive fibers from the olfactory bulb, which was well developed. In one series of experiments the olfactory placode was transplanted to a superficial position so that (presumably) it was capable, in later stages, of normal peripheral stimulation; in another series the placode was buried beneath the skin so as to preclude stimulation from the exterior. The related transplanted hemispheres developed equally in the two cases, showing that the stimulus to differentiation of the olfactory centers is provided by the ingrowth of the olfactory fibers, even though these are never normally excited. It would be interesting to learn the effect of carrying the experiment further and transplanting the cerebral hemisphere alone without the olfactory placode, thus isolating it completely from any form of nervous excitations. This would show whether the primitive hemisphere vesicle has any capacity whatever for intrinsic differentiation beyond the stage normally reached when nerve fibers first penetrate it from adjacent parts.

In another series of experiments it was shown that extirpation of the hemispheres in prefunctional stages is followed by very

little regeneration in case the olfactory placode is also removed; but if the latter is left in place the hemisphere does regenerate to normal form under the influence of the ingrowing olfactory fibers.

Such experiments can be indefinitely multiplied, and it is very desirable that this should be done, and also that after such operations the larvae should be reared (in so far as they are viable) to a sufficiently advanced stage of functional development as to permit of precise determination of the regulatory changes in the arrangement of the fiber tracts and correlation centers by comparison with normal larvae of corresponding ages. This can be accomplished with considerable completeness by the use of the silver-impregnation methods of Cajal and Bielschowsky. Normal specimens of various ages up to adult are already available in the collections of Coghill, McKibben, Watkins, Burr, and others, and these are now in process of intensive study.

It is no accident that these and many other energetic workers in so diverse fields have converged their activities upon the development of the Amphibia. This material is very favorable for attack upon the most fundamental problems of growth and differentiation. In the cerebral hemispheres we find here the same morphological type as in mammals, but reduced to lowest terms, and the morphogenetic factors in the two cases doubtless have much in common. Studies on the early development of the human cerebral hemispheres made by Dr. Marion Hines Loeb and others in the laboratories of the University of Chicago have brought to light unexpected similarities with those of lower vertebrates, features which have in the past been overlooked just because the simpler paradigm was not taken into the reckoning.

The various researches to which reference has been made here and others on kindred topics which might have been mentioned have, for the most part, been independently planned and executed. They represent no formal collaboration, no elaborate machinery of organization. Men of diverse training and aptitudes have studied problems as they were presented, and their natural interests have drawn them together from different quarters. In the aggregate these investigations form an impressive

exhibit of successful accomplishment and still greater promise for the future.

As for this future, the best results will probably continue to follow from the unrestrained initiative of the individual workers. But, in view of the fact that this entire body of research does have so obvious an application to fundamental problems, and that the concentration of so diverse inquiries upon a single species gives broader promise than could be expected from any single line of investigation, it is probably expedient for those now working on *Amblystoma* to seek somewhat closer contacts with each other and more consciously so to direct their programs of research as to make the largest possible use of data supplied by their co-workers.

In particular, the experimental workers, who may not be in a position to carry to completion the very desirable (but very laborious) morphological studies necessary for the broadest interpretation of their results, may find it advantageous at times to seek conference on these questions. I refer especially to matters of internal structure, courses of fiber tracts, functional connections of correlation centers, etc., at definite ages, regarding which there is a large body of ascertained fact, much of which is as yet unpublished.

One of the first desiderata is an authoritative record of the chief characteristics of the nervous system at successive stages of development, arranged preferably by stages, as yet unpublished, as defined by Harrison, and these in correlation with known stages of physiological development—in short, a series of normal tables of the nervous system, based on the sequence of both structural and functional development. This enterprise it is hoped will soon be undertaken in the Yale laboratories under the direction of Doctor Burr, and will serve as a useful norm or datum of reference for comparison with the quantitative studies of growth of mammalian types now so actively prosecuted in several American laboratories.

An account of the plastic changes in the external form should follow, with pictures of the stages from models. The description of these form changes is, however, largely dependent for its value

upon the identification of the regions in correlation with adult structure and functional localization at successive ages. The data for such a correlation are still far from complete, but are accumulating, and it is hoped that the time is not far distant when such a description can be written, with more or less of correlation of changes in external form with corresponding changes in behavior pattern and in histological differentiation internally. More detailed studies of the development of reflex patterns from early larval to adult stages are necessary for the consummation of the program.

March 2, 1922

#### ADDENDUM

At the time when these proof sheets pass to the press (April 11, 1922) it can be stated that the studies referred to in the last sentence above are in process. Under a research program organized jointly by the University of Kansas and the University of Chicago, Doctor Coghill and Mr. Watkins have this season already studied intensively the early development of the reflex pattern in upwards of 100 individual larvae of *Amblystoma* and the work is now continuing to include still other series of specimens. Many hundreds of specimens of tested physiological age have been fixed for future histological study.

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Resumen por el autor, Austin T. Moore.

Una conexión anómala de los músculos piriforme y biceps  
del fémur.

Durante una disección de un varón negro bien desarrollado, de unos treinta y siete años de edad, el autor encontró un músculo distinto biventral que se extendía desde la pelvis llegando hasta el biceps femoral, en la unión de sus cabezas larga y corta. El músculo se originaba en forma de un tracto separado de las fibras antero-inferiores del músculo piriforme, pasando a través del gran foramen ciático y procediendo distalmente y lateralmente sobre la superficie posterior de la tibia por debajo del glúteo máximo. Un poco antes de alcanzar el borde inferior de este musculo se convertía en tendinoso, continuando de este modo hasta el nivel del origen superior de la cabeza corta del beceps. En este punto el musculo descrito volvía a transformarse en carnoso terminando el en biceps femoral, en la unión de sus cabezas corta y larga.

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## AN ANOMALOUS CONNECTION OF THE PIRIFORMIS AND BICEPS FEMORIS MUSCLES

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### ONE FIGURE

During the course of dissecting a well-developed negro, apparently between thirty-five and forty years of age, there was found a distinct double-bellied muscular anomaly in association with the piriformis and biceps femoris muscles, extending from the origin of the piriformis to the junction of the long head of the biceps with its short head. No other muscular abnormalities were observed.

The muscle arose as a detached slip of the lower anterior fibers of the piriformis, and lay on the anterior surface of this muscle near its lower border in the first part of its course. Passing out of the greater sciatic foramen, it proceeded distally and laterally in the posterior aspect of the thigh under cover of the gluteus maximus. Just before reaching the lower border of this muscle it became tendinous. This tendon, of approximately 2 mm. in diameter, continued underneath the long head of the biceps till it reached the level of the upper origin of the short head of the biceps. Here the muscle again became fleshy and continued to the junction of the long and short heads of the biceps, terminating in their common tendon of insertion into the tibia and fibula. The general appearances and relations are diagrammatically shown in the accompanying figure.

The superior belly derived its nerve supply from a small branch of the great sciatic nerve. The inferior was supplied by two small twigs from the branch of the nerve to the hamstring muscles that supplied the short head of the biceps.

The blood supply was derived from two sources. A branch of the inferior gluteal artery supplied the superior belly, while the inferior belly was supplied by twigs from the third and fourth perforating branches of the profunda femoris artery.

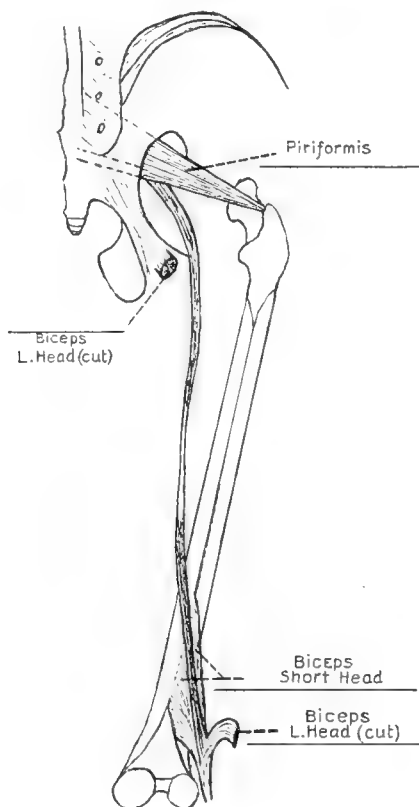


Fig. 1 Sketch of a double-bellied anomalous muscle extending from the piriformis to the junction of the long and short heads of the biceps. From an adult negro.

In the literature accessible to me, no anomaly identical with this has been found, although several observers have reported muscular fascicles, associated with the biceps, with which the muscle herein reported has some common resemblances.

Wood (1) reports a muscle arising from the under surface of the gluteus maximus and inserted into the long head of the biceps.

Halliburton (2) describes a muscle having a contiguous origin with the long head of the biceps, but inserted into the gastrocnemius.

Testut (3) reports a double origin of the biceps, the abnormal head arising from the coccyx.

LeDouble (4) reports a muscle having a common origin with the biceps, but inserted into the tendo calcaneus. He also states that Macalister has reported a muscle arising from the outside of the sacrum and that Soemmering has reported one arising from the pelvis, but terminating at the ischium.

LeDouble, from consideration of the abnormalities of the biceps observed by himself and also from the reports of others, divides them into two classes: First, those having a pelvic origin terminate in the long portion of the biceps; second, those having an origin in the posterior region of the thigh terminate in the short portion of the biceps. It is seen that the muscle here reported falls in neither of these classes.

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Resumen por el autor, O. Ishii.

### Observaciones sobre el ciclo sexual de la rata albina.

En el presente trabajo se discuten los siguientes puntos: 1) ¿Sigue la preñez a la primera copulación de la rata? 2) Duración del periodo de gestación. 3) Influencia de la alimentación sobre el número de individuos de la cría. 4) Condición de la rata en vías de crecimiento después del nacimiento. 5) La época del primer estro general en la rata. 6) Signos del primer estro general. 7) Distinción entre el estro general y el periodo de ardor sexual propiamente dicho. El autor ha encontrado que el método de los frotos no permite una distinción entre estas dos condiciones. 8) Duración, época de la aparición y signos del periodo de ardor sexual propiamente dicho en la rata. 9) Periodo diéstrico. 10) ¿Ejerce la copulación estéril influencia sobre el ciclo sexual de la rata?

Translation by José F. Nonidez

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## OBSERVATIONS ON THE SEXUAL CYCLE OF THE WHITE RAT

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1. Normal pregnancy does not usually result from the first copulation, but only when copulation has taken place more than once does the female become pregnant. For this purpose it is, furthermore, necessary that copulation with a healthy male take place at the actual period of heat of the female rat. On very rare occasions, however, pregnancy may result from a single copulation. The same I found also to hold good in the case of guinea-pigs.

2. In the majority of cases the duration of the gestation period of the rat is  $21\frac{1}{2}$  to  $22\frac{1}{2}$  days. My observations on this point confirm those of Stotsenburg (4). However, in some exceptional cases I have found the period of pregnancy to last 23 days.

3. To determine the influence of nourishment on the size of the litter, I observed two sets of ordinary stock rats. a) In the first set they were fed grain, green vegetables, and milk once a day; b) in the second set they were fed table scraps three times a day. This material was fresh and in good condition every time. In both sets special care was taken to keep the cages dry, since wet cages disturb the health of the animals. In the first set the number in the litter averaged from six to nine and sometimes reached ten or eleven; in the second set the average was greater, namely, from nine to twelve and sometimes more. Stotsenburg (4) also found that feeding with table scraps causes the size of the litter to be larger than a diet of bread and milk.

4. The weight of the young rat at birth was on the average from 4 to 5 grams; this agrees with the statements of various authors. In addition, I have observed that the hair begins to grow about seven to eight days after birth, when it has the

appearance of very fine fur visible to the naked eye. In healthy rats the eyes open about on the fourteenth or fifteenth day.

5. The time of the first general oestrus in the rat. The statements of various authors vary as to the age at which the first general oestrus occurs in the rat.<sup>1</sup> Thus Long and Evans (3) state that on the average it takes place at the age of 70 to 90 days and in some cases the date may vary between 45 and 120 days, while Donaldson reports that breeding begins at the age of about three months. Recently H. M. Evans and K. L. Bishop stated that the first oestrus begins on the average at the age of 47 days and that the dioestrous period lasts on the average of 5.4 days. This holds good if the animals are fed with a standard diet consisting of whole wheat, whole-milk powder, casein, salts, and 5 per cent butterfat. According to my statistics, in rats which are fed on table scraps the opening of the vagina or the first menstruation occurs in normal animals weighing from 60 to 80 grams at an age of 32 to 40 days; sometimes they may, however, be as old as 45 days.

6. Signs of the first general oestrus. Up to the time of the first general oestrous period the vaginal lips are closed with a thin membrane. Gradually the membrane begins to rupture. The maximum enlargement in the opening is reached five to seven days after the onset of the general oestrus. During this period there is no appreciable amount of moist secretion visible at the vaginal orifice; only a dry material is found. This is followed by a stage in which the secretion becomes more copious and serous or watery in character as it is found at the ordinary oestrous period. Now, the vaginal lips are slightly swollen and glistening. In some cases the rat comes into actual heat in the first general oestrous period, but often copulation does not take place at that time. In other words, the animal has not yet reached the condition of heat proper. After the onset of the second general oestrous period real heat occurs in every rat. According to my previous

<sup>1</sup> We shall distinguish between general oestrus and heat proper. By the former we understand the whole period during which an increased secretion is visible at the vaginal orifice, while the latter is only the period in which the female is ready for copulation. The second period forms a part of the first one.

observations, conditions are analogous in the guinea-pig. Here also the first general oestrus is prolonged.

7. Distinction between heat and the general oestrus in the rat. Some authors do not distinguish between the period of general oestrus, which is characterized by secretion, and the heat proper. When the rat comes into heat, she becomes somewhat more playful and holds up her tail and the external portion of the vagina. At this time she is ready for copulation, if a male rat approaches her and attaches himself to her back. Whenever she is not ready for copulation, the real period of heat is not present. The heat represents one period in the general oestrous period. Long and Evans have stated that they can determine the heat period by means of the vaginal-smear method of Stockard and Papanicolaou (5). Contrary to this conclusion, we do not find that the smear method enables us to distinguish between heat proper and the general oestrous period.

8. The heat period proper in the rat. This period has an average uninterrupted duration of from four to six hours. During the greater part of the period there is a secretion visible on the external vagina. Towards the end of this heat period, however, the secretion is either lacking altogether or its amount is very much decreased. I have found this to hold equally good in the case of the guinea-pig. In one exceptional case I have, however, noticed that the heat period proper lasted continuously for eight hours. Another important point is that in most cases the rat enters into the heat period proper usually about midnight, or after 9 o'clock; only in rare cases does this period take place in the daytime.

*Duration of the dioestrous period in the rat*

NUMBER OF ANIMAL	AGE AT TIME FIRST OESTRUS	1ST	2ND	3RD	4TH	5TH	6TH	7TH	8TH	9TH	10TH	11TH	12TH	13TH	14TH
	<i>days</i>														
1	33	11	18	5	5	7	3	8	5	3	6	7	4	5	8
2	35	4	5	13	10	5	9	5	20	3	4	2	4	2	4
3	43	5	5	12	6	9	3	5	4	3	2	5	4	3	6
4	45	4	4	12	10	15	4	9	5	3	5	11	2	7	5

The figures signify the number of days between two successive oestrous periods.

9. Dioestrous period of the rat. According to Long and Evans (3), the rat shows a very peculiar dioestrous period. The length of this period varies in each individual case. We likewise obtained a marked variation in the length of the dioestrous period.

According to the data of this table, the longest duration of the dioestrous period was twenty days; the shortest two days; furthermore, in each individual the duration varies in different periods. There is some reason for assuming that in case the dioestrous period lasts only two days heat proper did not develop inasmuch as the female did not accept the male, although vaginal secretion was present. True heat was, however, found in those rats in which the dioestrous period lasted three or more days.

10. Does sterile copulation exert an influence on the sexual cycle in the rat? According to Long and Evans (3), a sterile copulation in the rat leads to a lengthening in the subsequent dioestrous periods. Some years ago I had made a similar suggestion; however, actual observation of guinea-pigs, in which the animals after copulation apparently did not develop pregnancy, did not reveal any difference in the duration of the sexual cycle between these and normal animals which had not copulated. My recent observations show that the same holds good in the case of the rat. Here also sterile copulation has no influence on the sexual cycle.

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## Resumen por el autor, Howard H. Bell

### Páncreas horizontal y vertical en asociación con otras anomalías del desarrollo.

El primer ejemplar descrito posee un proceso horizontal de 51 mm. de longitud, semejante al páncreas normal, un proceso vertical de 28 mm. de longitud, que pasa hacia abajo deslizándose sobre la superficie mesial del duodeno, y un proceso anular alrededor de este último; todos estos procesos se reúnen en el sitio ocupado por la cabeza del páncreas normal. El proceso anular presenta un conducto separado. El tabique interventricular del corazón es defectuoso por debajo de la parte membranosa. El agujero oval y el conducto de Botal son permeables. En el lado derecho existe una hernia umbilical y otra inguinal. El divertículo de Meckel persiste.

En el segundo ejemplar ha hallado el autor: Un divertículo de la pared posterior del estómago, situado debajo del orificio esofágico y unido con las meninges a través de las hendiduras vertebrales. El intestino delgado abandona la pared posterior del estómago en la línea media, debajo de este divertículo. En el extremo pilórico del estómago existe otro divertículo, origen del hígado y páncreas. El páncreas posee un proceso horizontal de 65 mm. de longitud, el cual cruza la pared anterior del estómago, y un proceso vertical de 34 mm. que pasa hacia abajo. El primero posee un conducto independiente que se abre en el divertículo a una distancia de 5 mm. del otro orificio. Resumen de las anomalías asociadas: Espina bífida posterior con mielocistomeningocele; espina bífida anterior; puente intraespinal, médula oblongada y cerebelo rudimentario; ausencia del tentorio cerebelar; ausencia de la isla de Reil; ausencia de glándula pineal, acueducto de Silvio y cuerpos cuadrigéminos; anormalidad en la circulación cardiaca, hígado, estómago y páncreas; defectos en el lado izquierdo del diafragma; ausencia del omento mayor, divertículo de Meckel bifurcado; disposición anormal de los intestinos grueso y delgado; testículos no descendidos; talipes varus.

## HORIZONTAL AND VERTICAL PANCREAS, IN ASSOCIATION WITH OTHER DEVELOPMENTAL ABNORMALITIES

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TWO FIGURES

This type of abnormality in the development of the pancreas was described by Carnot.<sup>1</sup> The pancreas in his case possessed a head, a horizontal process 10 cm. long, resembling the body and tail of the normal pancreas, and a vertical process 12 cm. long extending downward from the head along the duodenum. Neve<sup>2</sup> mentioned extra tail lobes as an instance of reversion to a lower type. I have failed to find further reference in the literature to pancreas with horizontal and vertical processes, yet I have found two instances of this anomaly.

The first instance occurred in a well-nourished white male child, four months old. The cause of death was bronchopneumonia and extensive atelectasis.

The pancreas completely encircled the second part of the duodenum and possessed a horizontal and a vertical process. The band of pancreatic tissue encircling the duodenum was 10 mm. wide anteriorly, and 6 mm. wide posteriorly. The horizontal process resembled the normal pancreas. It measured 51 mm. long and 11 mm. in the other dimensions. Along the upper border near the duodenum was a rounded enlargement 11 mm. across. The vertical process descended along the mesial surface of the second part of the duodenum and passed anterior to the transverse part. This process measured 28 mm. long and 9 mm. in the other dimensions. The two processes and the annular part joined at the site occupied by the head of the nor-

<sup>1</sup> Carnot. *Maladies des glandes salivaires*. Paris, 1908.

<sup>2</sup> Neve. *The Indian Medical Record*, 1892, vol. 3, no. 3.

mal pancreas. The annular part encircled the duodenum from behind forward and was in apposition with pancreatic tissue anteriorly, but not continuous with it. The duodenum was slightly constricted at this point.

The common bile duct opened into the duodenum with the pancreatic duct from the horizontal and vertical processes. The annular part possessed an individual duct opening into the duodenum slightly above and posterior to the ampulla of Vater.

Other developmental abnormalities were present. The interventricular septum of the heart was defective below the membranous part. This defect measured 21 mm. vertically, and 11 mm. horizontally. The foramen ovale and ductus Botalli were pervious, but the openings were very small. There was a slight umbilical hernia and a small inguinal hernia on the right side. Meckel's diverticulum persisted and measured 5 cm. long.

The second anomaly occurred in association with anterior spina bifida and other abnormalities which will be described elsewhere.

The infant was still-born at term. The stomach was greatly dilated. The small intestine joined the posterior wall of the stomach in the midline of the body. It opened into the cavity of the stomach by a slit-like orifice guarded by a valve-like fold. From the point where the intestine met the stomach to the site of its gastric orifice, it lies beneath the gastric peritoneum (See Fig. 2). The gastric orifice of the intestine was 5.5 cm. from the pyloric end of the stomach. The pyloric end of the stomach had the form of a blind diverticulum into which the common bile duct opened. The horizontal and vertical pancreatic processes met upon this diverticulum. The diverticulum was about 1 cm. wide and 1.5 cm. long and ended in a blind rounded extremity.

The pancreas possessed a horizontal and a vertical process. The horizontal process extended across the anterior wall of the stomach and was loosely attached to it. It measured 65 mm. long, 9 mm. wide, and 4 mm. thick. The vertical process extended downward. Its lower extremity was slightly enlarged and was turned abruptly backwards. The upper part of this process measured 34 mm. long, 9 mm. wide and 4 mm. antero-

posteriorly. The lower part, which was enlarged and turned backwards, measured 12 mm. anteroposteriorly, 14 mm. vertically, and 5 mm. transversely. The horizontal and vertical processes were contiguous over the summit of the diverticulum.

There were two papillae with openings into the diverticulum, situated about 5 mm. apart. The duct from the vertical process led in conjunction with the common bile duct into the end of the diverticulum. The duct from the horizontal process opened into the superior wall of the diverticulum.

The relation of the pancreatic processes and associated organs to the stomach is shown in fig. 1.

Many abnormalities were associated with the second specimen. The liver was abnormal in shape. There was a funnel-shaped diverticulum from the posterior wall of the stomach below and posterior to the oesophageal opening, which was attached to the meninges through the anterior spina bifida. The relation of this opening to the stomach is shown in fig. 2.

A summary of associated abnormalities is as follows:

Posterior spina bifida with myelocystomeningocele

Anterior spina bifida

Intraspinal pons, medulla and rudimentary cerebellum

Absence of tentorium cerebelli

Accessory superior longitudinal fissure in right hemisphere

Absence of islands of Reil

Absence of pineal body, aqueduct of Sylvius and corpora quadrigemina

Abnormality of heart

Defect in diaphragm on left side

Abnormality of liver and stomach

Absence of greater omentum

Bifurcated Meckel's diverticulum

Abnormal arrangement of small and large intestines

Undescended testicles

Talipes varus.

Two related cases described by Graefe and Vesalius were mentioned by Mondière.<sup>3</sup> The one described by Graefe pos-

<sup>3</sup> Mondière. *Archiv. gén. de Méd.*, 1836, 2d ser., T. 11.

sessed three pancreatic ducts, one of which opened into the stomach. Richeraud stated in his physiology that Vesalius described a case in which the ductus choledochus emptied into the stomach.

Aberrant pancreatic tissue has occurred in the wall of the stomach in several instances.<sup>4</sup>

The human pancreas develops from one posterior and one anterior bud; occasionally there are two anterior buds which soon fuse to form one. The posterior and anterior processes grow together to form the mature pancreas.<sup>5</sup>

The shape of the pancreas varies in different species. The pancreas of the ox and sheep is irregularly quadrilateral in form.<sup>6</sup> The pancreas of the horse is very irregular, although triangular in outline. The pancreas of the pig is elongated and situated transversely. The right portion has two lobes; the posterior lobe bends around the great mesenteric artery and the portal vein; the anterior one is pointed, extends downward and forward in the median plane and is attached in the portal fissure and to the duodenum. The left portion is prismatic and rests mainly on the left sac of the stomach, its left extremity being included between the left kidney and the dorsal end of the spleen. The pancreas of the dog is V-shaped, consisting of two long, narrow branches which meet at an acute angle. The right branch extends along the duodenum. The left branch is attached to the peritoneal surface of the stomach. In the cat the pancreas has two processes; one process extends along the duodenum and the other process extends along the stomach. The two processes are nearly at right angles to one another.<sup>7</sup>

<sup>4</sup> Opie, E. L. Disease of the pancreas. 1910. J. B. Lippincott Co., Philadelphia.

<sup>5</sup> Keith. Human Embryology. 1913. Edward Arnold, London.

<sup>6</sup> Sisson. Veterinary anatomy. 1910. W. B. Saunders Company, Philadelphia.

<sup>7</sup> Reighard and Jennings. Anatomy of the cat. 1910. Henry Holt & Co., New York.

The first specimen resembles the pancreas of the dog and cat in that it has a horizontal and a vertical process, although it has in addition an annular process. The position of the pancreatic ducts in this specimen would indicate that the annular part developed from posterior budding and the horizontal and vertical processes from anterior budding.

The second specimen was associated with many unusual abnormalities. The horizontal and vertical processes were in apposition on the summit of the gastric diverticulum, although no fusion had taken place.

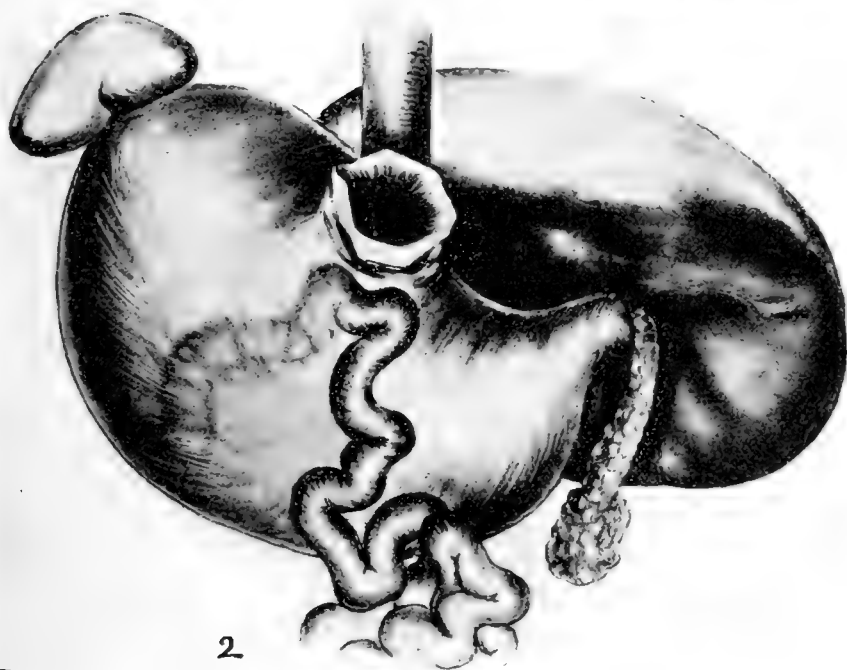
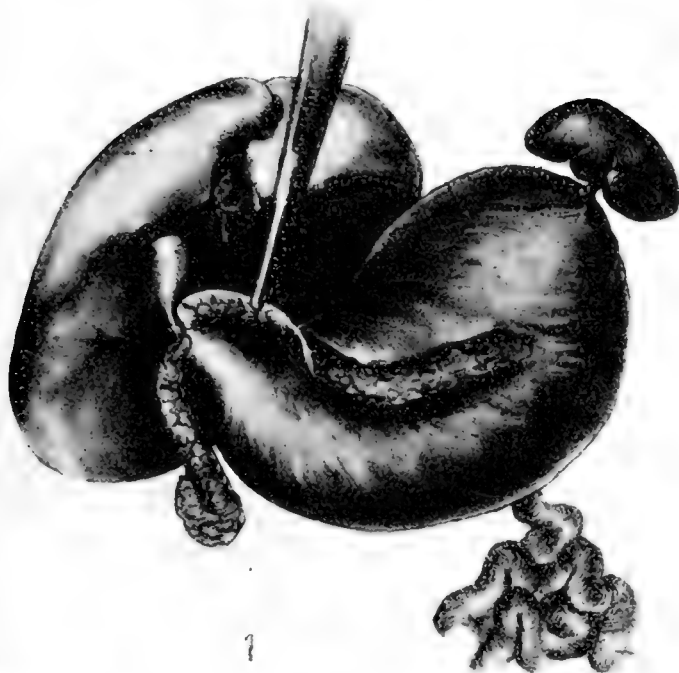
## PLATE 1

### EXPLANATION OF FIGURES

1 Drawing of anterior view of liver, stomach, pancreas and spleen. The liver is raised, and the right end of the horizontal pancreas is elevated with forceps, to show the relation of the liver, the horizontal and vertical pancreas to the diverticulum of the stomach.

2 Drawing of posterior view of organs. The liver is tilted forward. Below and posterior to the oesophagus is shown the diverticulum which was attached to the cleft vertebrae. Below this diverticulum the small intestine passes obliquely through the wall of the stomach and opens into it.





## BOOKS RECEIVED

(Continued from first page)

THE RANSOHOFF MEMORIAL VOLUME. A Collection of Papers representing Original Contributions to the Art and Science of Medicine by Colleagues and Students of Dr. Joseph Ransohoff, M.D., F.R.C.S. (Eng.), F.A.C.S., LL.D. 574 pages, illustrated, containing 57 papers. Cincinnati, Ohio, S. Rosenthal & Co. 1921. A list is given of the 44 papers published by Dr. Ransohoff between the years 1879 and 1920. *From the Editors' Preface.* In an effort to express to Dr. Joseph Ransohoff their appreciation of his place in the science and art of Medicine, a group of his former students determined to issue a volume containing papers consisting of original contributions to the advancement of Medicine by his students and colleagues. This was in June, 1920. The death of Dr. Ransohoff prevented the presentation of this book to him personally, but the publication was continued as a Memorial Volume.

THE RELATIVE VALUE OF THE PROCESSES CAUSING EVOLUTION by Arend L. Hagedoorn, Ph.D. and A. C. Hagedoorn—Vorstheudel La Brand, with twenty figures, 294 pages. The Hague, Martinus Nijhoff, 1921, printed in the English language. The Chapter headings are: Introduction, Heredity, Variation, Crossing, Reduction of Variability, Mutation, Selection, Species and Varieties, The Law of Johannen, Evolution in Nature and under Domestication, The Status of Man.

## BOOKS RECEIVED

**A MANUAL OF STYLE**—A compilation of typographical rules governing the publications of The University of Chicago, with specimens of types used at the University Press, Seventh Edition, 300 pages, size of page 5 x 7½, Price \$1.50, November, 1920, The University of Chicago Press, Chicago, Ill.

Contents: Rules for Composition (capitalization, the use of Italics, quotations, spelling, punctuation, divisions, footnotes, indexing, tabular work). Technical Terms. Appendix (hints to authors, to proofreaders, to copyholders, proofreaders' marks). Specimens of Types. Index.

The first edition was issued in 1906, having been compiled from proofreaders' notes "jotted down by the first proofreader at odd moments for his own guidance; added to from year to year as opportunity offered or new necessities arose; revised and re-revised as the scope of the work, and, it is hoped, the wisdom of the workers increased—it emerges in its present form, . . . the blended product of the reflections of many minds."

The fourth edition (1913) was entitled, "A Manual for Writers, Covering the needs of authors for information on rules of writing and practices in printing."

A book has recently been issued by *The Atlantic Monthly* of the same size page and covering the same subjects, the result of a similar evolution.

**MEDIZINISCHE PSYCHOLOGIE**, Ein Leitfaden für Studium und Praxis, von Dr. Ernst Kretschmer, Privatdozent für Psychiatrie in Tübingen. Leipzig, 1922, Georg Thieme Verlag—size 5 x 8, 306 pages.

**ANXIETY HYSTERIA**, Modern View on Some Neuroses, by C. H. L. Rixon, M.D., M.R.C.S. and D. Matthew, M.C., M.B., Ch.B., with a Foreword by Col. Sir A. Lisle Webb, 124 pages, 4¼ x 7¼, price \$1.50, Paul B. Hoeber, New York, 1921.

**SEPARAT-ABDRUCK** aus der Zeitschrift für Morphologie und Anthropologie, Band XXII, Heft 1 und 2. Der Menschenfuss von Dr. Franz Weidenreich, a.o. Prof. der Anatomie z. Z. Mannheim. Stuttgart, 1921. E. Schweizerbart'sche Verlagsbuchhandlung. Size 6½ x 9½, 282 pages.

Resumen por John L. Bremer, por el autor, Kikuo Okamoto.

Un estudio de las venas superficiales de la extremidad superior  
en sujetos japoneses vivos.

El presente trabajo es una descripción de las venas superficiales del brazo en una serie de 200 varones japoneses, habiéndose notado diversos tipos de variaciones. Comparadas con cuadros de variaciones semejantes encontradas en europeos, puede notarse un tanto por ciento distinto de diferencias.

Translation by José F. Nonidez  
Cornell Medical College, New York

## A STUDY OF THE SUPERFICIAL VEINS IN THE SUPERIOR EXTREMITY OF LIVE JAPANESE

KIKUO OKAMOTO

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TEN FIGURES

A study of the superficial veins in the superior extremity of live subjects is important for intravenous injection in practice. With this object, I have examined the superficial veins in the superior extremity of live Japanese. A similar investigation we have already seen in the paper of Richard J. A. Berry and his student, H. A. S. Newton.<sup>1</sup> But this study of Japanese is not without interest for the anatomy of the race. I shall be very happy if this study proves of service to the angiology in Japanese, which is now in the hands of my teacher, Prof. Dr. B. Adachi (Kyoto).

The subjects of my study were one hundred men. Eighty of these were students of the Medical Department of the Keio University and twenty were my friends, for which I express my sincere gratitude for their kindness. The procedure adopted, as Berry and Newton used, was the usual one of keeping the limbs in a dependent contraction and then banding the distended veins.

### *A. The v. cephalica (B.N.A.)*

In the paper of Berry and Newton we seen the v. cephalica arising as a direct communication of the radial end of the arcus venosus dorsalis manus. But in my case I have found it arising from the v. metacarpea dorsalis I by the rete venosum dorsale manus. Of the proximal course of this vein occur three types.

<sup>1</sup> Richard J. A. Berry and H. A. S. Newton. A study of the superficial veins of the superior extremity in 300 living subjects. *Anat. Anz.*, Bd. 33, 1908.

Type 1. The *v. cephalica* curves round the radial border of the forearm proximal to the *processus styloideus* of the radius on the volar surface. Here the vein continues to pass proximally and slightly medially to the bend of the elbow, where it gives off the *v. mediana cubiti*. Continuing its proximal course, the *v. cephalica* runs in the *sulcus bicipitalis lateralis* and then in the *trigonum deltoideopectoriale* to its termination. This first type in my description has no accessory cephalic vein and no 'Inselbildung.' I have found this type in 19 per cent in my cases (21 per cent on the right, 17 per cent on the left).

Type 2. The *v. cephalica* has a so-called 'Inselbildung' in its course. Berry and Newton did not describe such a type in their paper, but they described it in the *v. cephalica accessoria* which originated from the *v. cephalica* itself. They found this type in 16 per cent of cases in Europeans. I have found this in 27 per cent in Japanese (31 per cent on the right, 23 per cent on the left).

Type 3. The *v. cephalica* with an accessory vein (the *v. cephalica accessoria* B.N.A.). This accessory vein is a second longitudinal vessel situated laterally on the forearm, which opens into the main cephalic vein. In this case the main cephalic vein lies somewhat more medially than in the case without this vein. In Europeans Berry and Newton found this in no less than 80 per cent, but in Japanese I have found only 43 per cent (47 per cent on the right, 39 per cent on the left).

The termination of the *v. cephalica* was constant. Cases where the *v. cephalica* turned medially across the distal third part of the arm to terminate in the *v. basilica*, as described in the paper of Berry and Newton, were not found in my subjects. But cases in which the branchial portion of the cephalic vein was very weak were found in 4.5 per cent in my case (2 per cent on the right, 7 per cent on the left). In every instance Berry and Newton found the cephalic vein arising as a direct continuation of the radial end of the *arcus venosus dorsalis manus*. In my case it was abnormal in 6 per cent (5 per cent on the right, 7 per cent on the left). The cephalic vein arose from the ulnar end of the *arcus venosus dorsalis manus* or the *rete venosum dorsale manus* and the radial end of the *arcus* or the *rete* passed proximally to

the forearm and terminated in the v. basilica in the elbow flexure. In these cases the v. mediana antibrachii terminated in this abnormal vein. The cephalic vein received a large oblique vein (v. obliqua) from the ulnar end of the arcus venosus dorsalis manus in 41 per cent of my cases. The connection between the v. cephalica and the deep brachial vein in the elbow flexure was found relatively rare in live subjects. I have found this only in five cases (two cases on the right, three on the left) in 200 subjects. In this case the v. cephalica usually did not give off the v. mediana cubiti. The v. mediana cubiti was absent also in 14.5 per cent (10 per cent on the right, 19 per cent on the left) of my cases. The abnormal reduplication of the cephalic vein was found only in one case (no. 26, student twenty-two years) (see Abnormalities).

*B. The v. basilica (B.N.A.)*

The course of this vein is remarkably constant. As regards the normal origin of this vein, I have observed that it arose from the v. metacarpea IV or the ulnar end of the arcus venosus dorsalis manus. In 44 per cent (44 per cent on the right, 44 per cent on the left) the v. mediana antibrachii terminated in this v. basilica, which is in agreement with the figures of Berry and Newton (43 per cent in their case). The termination of the v. basilica in my subjects was constant (i.e., perforated the fascia brachii in the sulcus bicipitalis medialis). The type mentioned by Carle,<sup>2</sup> where this vein terminated in the v. axillaris, was not found in my cases. Very rare variation of this vein is of some interest in the making of 'Inseln' in its course. I have observed this only in two cases out of 200 arms (all were in the right arm). The other variations appeared in two cases (one on the right, one on the left), where the basilic vein was very weak. But in this case the v. cephalica accessoria, arising from the ulnar end of the rete venosum dorsale manus, was very strong in compensation.

<sup>2</sup> Carle, Recherches sur la veine basilique. Application à la ligature de l'axillaire. Bull. et Mém. soc. anat. Paris, Année 75, Sér. 6, T. 2.

*C. The v. mediana cubiti (B.N.A.)*

This is the connecting vein which normally leads obliquely upward in the flexure of the elbow from the v. cephalica to the v. basilica and has an anastomosis with the deep veins. This vein was present in 85.5 per cent (89 per cent on the right, 82 per cent on the left), which corresponds with the study of Berry and Newton (84 per cent in their case). The cases where the v. mediana antibrachii terminated in the v. mediana cubiti were much more frequent than in Europeans. In 54.5 per cent (56 per cent on the right, 53 per cent on the left) I have found these types (43 per cent in Europeans, after Berry and Newton). The vein arose from the v. cephalica much more distally than usual in 3 per cent (on the right side only one case and on the left six cases). I have observed sometimes the abnormalities of the reduplication of this vein, i.e., in 8.5 per cent (10 per cent on the left, 7 per cent on the right). Absence of this vein was found much more often than its reduplication, i.e., in 14.5 per cent in my cases (11 per cent on the right, 18 per cent on the left).

*D. The v. mediana antibrachii (B.N.A.)*

The v. mediana antibrachii is found to be the main outlet of the rete venosum volare manus. It passes proximally along the ulnar side of the volar surface of the forearm to a variable termination in the vicinity of the elbow-joint. Sometimes it makes a loop in the vicinity of the v. mediana cubiti, which receives the v. mediana antibrachii. The termination of this vein in my case is as follows:

1. It terminated in the v. basilica, 44 per cent (44 per cent on the right, 44 per cent on the left).
2. It terminated in the v. mediana cubiti, 54.5 per cent (56 per cent on the right, 53 per cent on the left).
3. It terminated by dividing into the v. mediana basilica and the v. mediana cephalica only in two cases in my 200 subjects. This type was usually described as an M-shaped arrangement of the veins in front of the elbow.



4. It terminated in a loop which lies in the vicinity of the v. mediana cubiti. Each end of this loop opened in to the v. mediana cubiti or the ulnar one in the v. basilica, 13.5 per cent (16 per cent on the right, 11 per cent on the left).

#### *E. The veins on the dorsal side of the hand*

From the union of every pair of the arcus venosus digitalis arise four larger vv. metacarpeae dorsales. These form sometimes the rete venosum dorsale manus as in B.N.A. and sometimes the arcus venosus dorsalis manus as noted by Berry and Newton. The large vein passing from the center of the concavity of the arcus venosum dorsale manus proximally to terminate into the v. cephalica or the v. basilica is called the v. ascendens. The radial extremity of the arcus or of the rete venosum dorsale manus receives vv. digitales propriae of the index-finger, as well as both the similar veins of the pollux. The large vein issuing from the ulnar end of the arcus venosus dorsalis manus and passing obliquely proximally and radially to terminate in the v. cephalica is called the v. obliqua. In my case I have the result as follows:

1. The arcus dorsalis venosus manus, 70 per cent (75 per cent on the right, 65 per cent on the left).

2. The rete venosum dorsale manus, 30 per cent (25 per cent on the right, 35 per cent on the left).

3. Presence of the v. obliqua, 41 per cent (44 per cent on the right, 38 per cent on the left).

4. Presence of the v. ascendens, 27.5 per cent (23 per cent on the right, 32 per cent on the left).

#### *F. Abnormalities*

The abnormalities in the superficial veins of the superior extremity in my studies were as follows:

1. The reduplication of the v. mediana cubiti was found in 8.5 per cent (7 per cent on the right, 10 per cent on the left).

2. The v. mediana cubiti was absent in 14.5 per cent (11 per cent on the right, 18 per cent on the left).

3. Connection between the v. cephalica and the v. cephalica accessoria was found in 2.5 per cent (3 per cent on the right, 2 per cent on the left).

4. The v. cephalica did not give off the v. mediana cubiti 14.5 per cent (10 per cent on the right, 19 per cent on the left).

5. The v. cephalica arose from the ulnar end of the arcus venosus dorsalis manus or the rete venosum dorsale manus and the radial end of the arcus or the rete passed proximally to the forearm and terminated in the v. basilica in the elbow flexure. In these cases the v. mediana cubiti was sometimes absent and the v. mediana antebrachii terminated in this abnormal vein. I have found this abnormality in 6 per cent (7 per cent on the right 5 per cent on the left).

6. The connection between the v. cephalica and the v. mediana antebrachii was found in 4.5 per cent (4 per cent on the right, 5 per cent on the left).

7. The v. cephalica was very weak in 4.5 per cent (2 per cent on the right, 7 per cent on the left). This abnormality combined often with abnormality 5. The absence of the brachial portion of the v. cephalica, which was found by Berry and Newton, was not seen in my cases.

8. The abnormal connection between the v. basilica and the v. cephalica in the upper arm was found in 3 per cent (2 per cent on the right, 4 per cent on the left).

9. The loop in the v. mediana antebrachii was found in 13.5 per cent (16 per cent on the right, 11 per cent on the left).

10. In case no. 61 (student twenty-five years) I have observed a very rare abnormality. The v. cephalica arose from the ulnar side of the rete venosum dorsale manus and ran proximally and terminated in the trigonum deltoideopectorale. In the right arm, the radial end of the dorsal veins of the hand arose normally and in the middle of the upper arm turned obliquely to the fossa axillaris and the terminated in the v. axillaris. In the left arm the v. cephalica at the fore margin of the m. pectoralis major gave off an abnormal branch to the axillary vein.

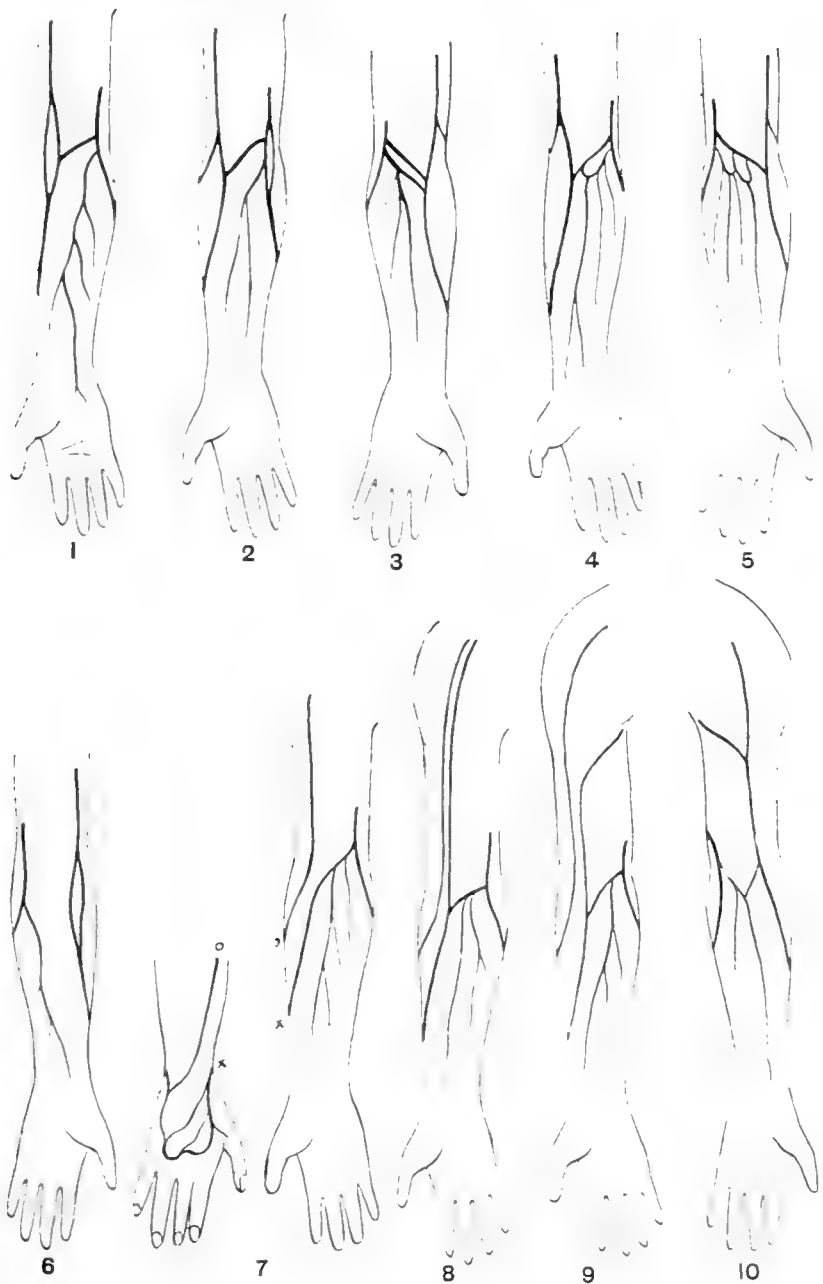
11. In case no. 26 (student twenty-two years) the v. cephalica in the right arm was duplicated. The lateral one arose from the v. ascendens.

12. In case no. 87. (student twenty-five years) I have found two loops in the flexure of the elbow in the left arm, in which vv. medianae antibrachii terminated.

## PLATE 1

### EXPLANATION OF FIGURES

- 1 No. 3 Twenty-four years old, right arm. The v. cephalica with 'Inselbildung.'
- 2 No. 11 Twenty-three years old, right arm. The v. basilica with 'Inselbildung.' The v. cephalica accessoria is present.
- 3 No. 29 Twenty-three years old, left arm. The duplication of the v. mediana cubiti is present.
- 4 No. 87 Twenty-five years old, right arm. The v. mediana antibrachii makes a loop in the vicinity of the elbow flexure.
- 5 No. 87 Twenty-five years old, left arm. The v. mediana antibrachii makes double loops.
- 6 No. 53 Twenty-six years old, left arm. The v. mediana cubiti is absent and the v. cephalica makes an 'Insel.'
- 7 No. 50 Twenty-five years old, right arm. The radial origin of the v. cephalica terminates in the v. basilica and the ulnar origin (corresponds with the accessorial origin) runs to the normal termination.
- 8 No. 26 Twenty-two years old, right arm. The reduplication of the v. cephalica is present, but the lateral one arises from the ulnar side of the dorsal veins of the hand.
- 9 No. 61 Twenty-five years old, right arm. The v. cephalica arises from the radical side of the dorsal veins of the hand and terminates in the v. axillaris. The lateral one arises from the ulnar side of the dorsal veins of the hand, and runs to the normal termination.
- 10 No. 61 Twenty-five years old, left arm. The v. cephalica gives an abnormal branch to the v. axillaris at the proximal third part of the upper arm. The v. mediana antibrachii divides into the v. mediana cephalica and the v. mediana basilica.



Resumen por el autor, William W. Looney.

Una vena espermática interna derecha aberrante.

En un sujeto mejicano, de unos veinticinco años de edad, fallecido de tuberculosis, ha encontrado el autor una vena espermática derecha aberrante la cual penetraba dentro de un hilus secundario situado cerca del polo inferior del riñón derecho, en compañía de una arteria renal accesoria, terminando en un vaso tributario de la vena renal. La relación de los vasos renales era también inversa de la normal.

Translation by José F. Nonidez  
Cornell Medical College, New York

## AN UNUSUAL ABERRANT RIGHT INTERNAL SPERMATIC VEIN

WILLIAM W. LOONEY

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### ONE FIGURE

The following observation was made on a Mexican male, about twenty-five years old, who died in Parkland Hospital of tuberculosis and was dissected during the first term of the present school year.

After the small intestine had been dissected and removed together with the mesentery, the peritoneum was stripped from the right psoas muscle. The right internal spermatic vessels were exposed just above the brim of the pelvis and traced upward. In following the vessels towards their origin and termination, it was found that the vein, instead of following the course of the artery, diverged to the right in the direction of the kidney.

On exposure of the hilus and lower pole of the right kidney, an accessory renal artery was found entering a distinct secondary hilus near the inferior pole of the kidney, accompanied by the internal spermatic vein. After removing a small portion of the anterior surface of the kidney (fig. 1), the internal spermatic vein was found to enter one of the tributaries of the renal vein.

It will also be noted from the figures that the ordinary relation of the renal artery and vein was reversed in this particular case.

Our recent texts and journals report cases in which the right internal spermatic vein enters the right renal vein, but none of them mentions cases parallel with the one described above.

Evidently, during differentiation of the kidney, the internal spermatic vein was included in the surrounding kidney substance and maintained in this position, thereby causing it to

terminate in a tributary of the renal vein, instead of in the main renal vein, as is ordinarily found in such cases.

It is interesting to note that had this particular kidney been removed during life, serious results would have followed in the right testicle, without the surgeon's being necessarily to blame.

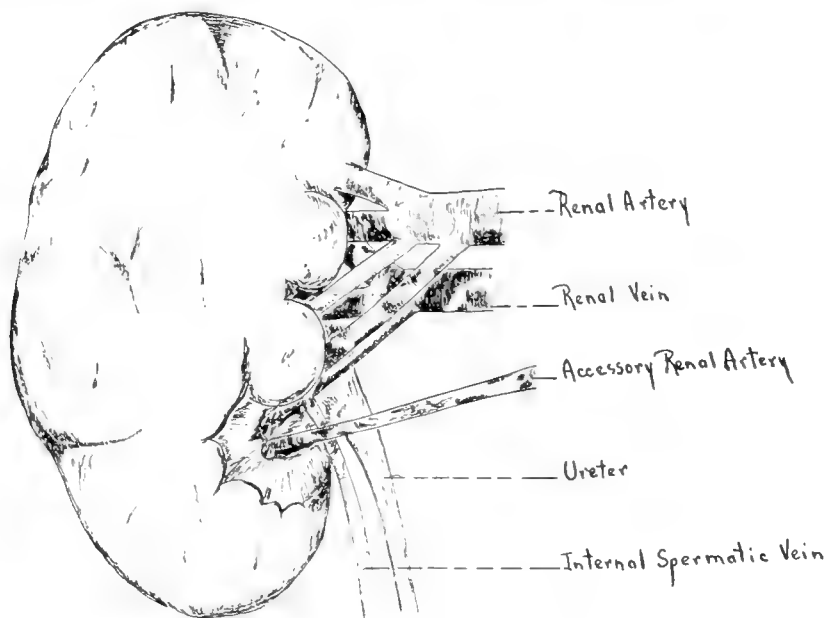
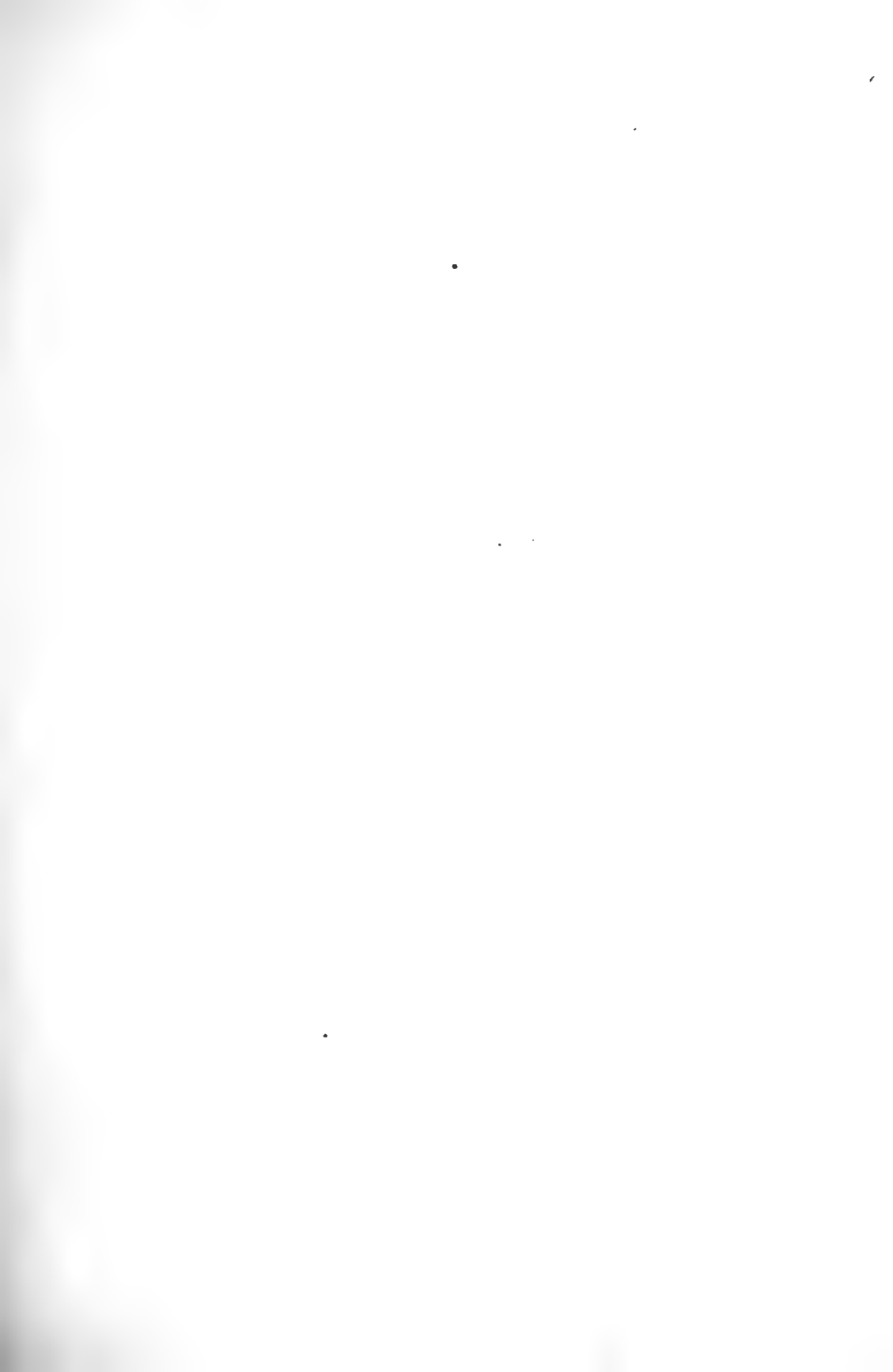


Fig. 1 Right kidney, with a portion near the lower pole cut away to show secondary hilus, accessory renal artery, and internal spermatic vein joining a tributary of the renal vein.





Resumen por el autor, George L. Streeter.

Algunas características uniformes de la oreja de los primates.

La parte articular, porción de la oreja destinada a su inserción a los lados de la cabeza, es una de las partes menos variables de la oreja de los primates. En general la parte articular corresponde a la mitad superior de la concha y comprende: a) Las eminencias articulares superior e inferior; b) la espina del helix; c) el pedunculo del helix. Las eminencias articulares superior e inferior ofrecen reunidas una superficie de inserción, en contacto directo con el cráneo. Están separadas por un pliegue de refuerzo el cual visto de lado se proyecta como el pliegue principal entre la fosa articular superior e inferior (fossa triangularis y cymba conchae) correspondiendo a las eminencias. El pedúnculo del helix forma el borde antero-lateral de la parte articular. Por encima soporta al helix y se continúa con él; por debajo marca la transición de la parte articular y la parte inferior de la concha.

Translation by José F. Nonidez  
Cornell Medical College, New York

## SOME UNIFORM CHARACTERISTICS OF THE PRIMATE AURICLE

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FOURTEEN FIGURES

In the attachment of the auricle to the side of the head there are certain physical requirements that must be met. The auricle must, of course, be held securely in place and at the same time it must possess a varying degree of motility and power of direction; its shape must be maintained for the efficient collection of sound vibrations, and provision must be made by some closure device to protect the external acoustic meatus from the entrance of foreign substances, particularly in burrowing and aquatic animals. In studying the striking differences in structure exhibited by the auricle in the various animal forms, one finds that most of their auricular individualities are in the nature of remarkable morphological adaptations to these varying physical requirements. Perhaps the most constant requirement is that the auricle be securely attached. In consequence one would expect to find that portion of the auricle concerned in this function to be less variable in form than its other parts. This appears to be true, at any rate for the Primates, and it is the purpose of this communication to call attention to this relatively constant articular provision and its expression in the form of the auricle.

The existing terminology of the external ear is a purely descriptive one and is based upon the form usually met with in the human adult. During the period of its inauguration scant attention was given to the embryonic stages and as little to the ear of other animals. It is therefore not surprising that one finds it more or less inadequate for any functional analysis of

the auricle or for the study of any ear other than that of adult man. When an appropriate time comes the nomenclature of the external ear, as much as any other part of the body, will need a thorough reconsideration. For the present, I am departing very little from the prevalent terminology, and then only where it seems unavoidable. As can be seen in figure 2, the following substitutions have been made: *fossa articularis superior* (for *fossa triangularis*), *fossa articularis inferior* (for *cymba concha*), *plica principalis* (for *crus inferius antheliceis*),<sup>1</sup> and *crus heliceis* to include all that part of the helix derived from the mandibular arch. Furthermore, on the median side of the cartilage, corresponding to the articular fossae, there are the superior and inferior articular eminences, partially separated by the groove of the principal fold. It is to these eminences that I would first of all direct the reader's attention.

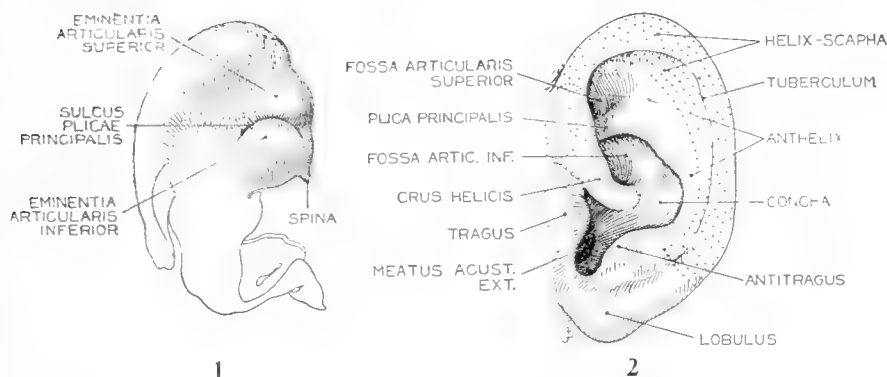
The two articular eminences (fig. 1) are continuous with each other anteriorly and together constitute a relatively rigid, bowl-shaped base from which the auricle is suspended. It is only this part of the auricular cartilage that offers a contact surface suitable for its attachment to the skull, and it may therefore be designated as the *pars articularis*. Nearly the whole of the inferior eminence contributes to this surface, whereas, of the superior eminence, only the forward and lower portions take part. It is true that the band-like, fenestrated cartilage surrounding the external acoustic meatus also has a bony attachment, but this is quite different in character and is to be compared rather to the tracheal rings, serving as a mechanism to prevent a collapse of the meatus. In structure and position it offers little if any support to the auricle.

The articular eminences are attached to the periosteum by a fibrous ligament sufficiently loose to permit free movement of the auricle. The extrinsic ear muscles are attached around the margins of the eminences. The superior auricular muscle is inserted into the superior eminence, the posterior auricular muscle into the conchal wall immediately adjoining the inferior

<sup>1</sup> I am adopting the term *plica principalis* as used by Boas (Ohrknopel und äusseres Ohr der Säugetiere. Kopenhagen, 1912).

eminence, while the anterior auricular muscle is inserted at the base of the spina. It is to be noted that the spina is in reality a process of the pars articularis, from that portion where the inferior articular eminence merges into the crus helix.

As for the crus helix, this is not, strictly speaking, a part of the helix, from which it differs both embryologically and structurally, as I have pointed out elsewhere.<sup>2</sup> It merges with the helix as a continuous fold, but one can always recognize the point of junction of the two at about the level indicated in

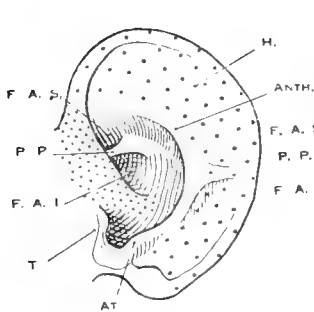


Figs. 1 and 2 Human adult auricle. In figure 1 the auricular cartilage is viewed from the median side, showing the two eminences that constitute its main area of contact with the skull. In figure 2 can be seen the cavities (fossae articulares) of these eminences with the plica principalis projecting between them as a strengthening ridge. The articular fossae are continuous with and constitute a specialized part of the concha, the pars articularis. The crus helix and the cartilaginous spina are also parts of this attachment mechanism.

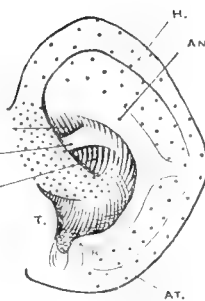
figure 2. The crus helix constitutes, first of all, the lateral rim of the bowl-shaped pars articularis and only secondarily acts as a support to the anterior end of the helix.

So much can be readily seen in the adult ear. In the human embryo and fetus the entity of a pars articularis is even more pronounced. It is the first part of the auricular cartilage to acquire its distinctive form and is more or less bowl-shaped from the outset, forming a cap over the upper end of the first

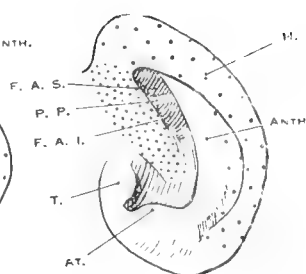
<sup>2</sup> Streeter: Embryological significance of the crus helix. *Anat. Rec.*, vol. 18, 1920.



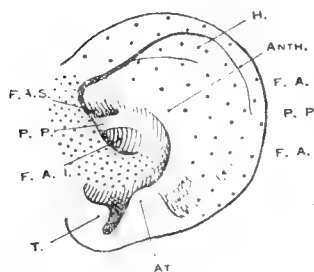
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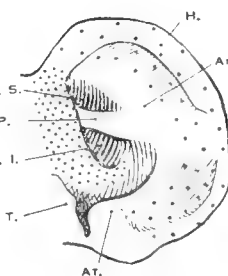
4. Gorilla



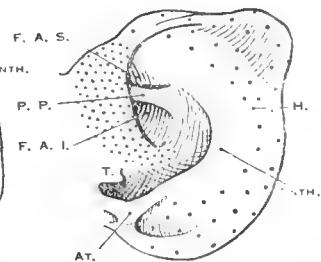
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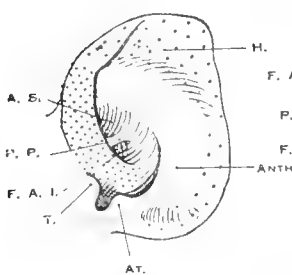
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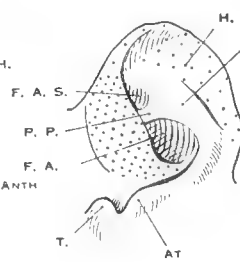
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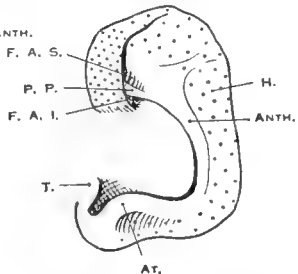
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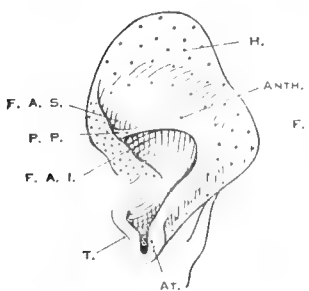
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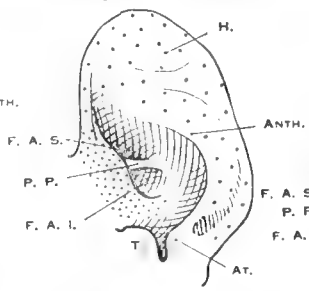
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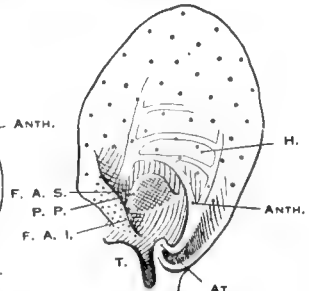
11. Howler



12. Marmoset



13. Lemur



14. Tarsius

Figures 3 to 14

gill cleft. It has a marked oral process (spina) extending forward into the mandibular arch and its lateral rim (crus helicis) is well defined. The floor, projecting against the skull, is early subdivided into two fossae by the plica principalis, which can be recognized in the 43-mm. fetus and is well pronounced in 50-mm. specimens. That portion of the floor corresponding to the inferior articular eminence is relatively larger, as compared with that of the superior, than obtains in the adult. The pars articularis is directly continuous with the concha and unquestionably should be considered a part of it. The latter spreads down towards the region of the antitragus and the meatus. In embryos less than 30 mm. long this portion of the concha is still fenestrated, which condition is more marked and remains permanent in the region of the meatus. The presence of the scapha, with its characteristic rolled edge (helix) is indicated early, but is relatively small and its growth slow until the later fetal stages.

Turning from these considerations to a survey of the auricle of other Primates, we find a striking constancy in the form of that portion concerned in its attachment—the pars articularis. A representative series of Primates is shown in figures 3 to 14. For convenience in arrangement, these figures are drawn at about the same size and so are at different enlargements. This treatment tends to minimize the marked differences in size actually prevailing in the scapha and helix in these different forms, and

Figs. 3 to 14 In these sketches the helix and scapha are dotted and the crus helicis is indicated by closer dots. Abbreviations: *ANTH.*, anthelix; *AT.*, antitragus; *F.A.I.*, fossa articularis inferior (cymba concha); *F.A.S.*, fossa articularis superior (fossa triangularis); *H.*, helix and scapha; *P.P.*, plica principalis (crus inferius anthelicis); *T.*, tragus. Most of these sketches were made from preserved specimens kindly lent to me by Dr. Adolph H. Schultz, and the species as identified by him are herewith given. (In the four specimens obtained elsewhere the source is mentioned.) Figure 3, *Troglodytes niger*, copied from figure V, page 214, of Boas ('12); figure 4, *Gorilla gorilla*, from photograph issued by the New York Zoological Society; figure 5, *Pongo pygmaeus*; figure 6, *Hylobates concolor*; figure 7, *Nasalis larvatus*; figure 8, *Pithecus philippinensis*; figure 9, *Papio porcarius*; figure 10, *Ateles variegatus*; figure 11, *Alouatta seniculus*; figure 12, *Hapale rufimanus*, copied from figure 243, Tafel 23, of Boas ('12); figure 13, *Lemur variegatus*; figure 14, *Tarsius spectrum*, from specimen belonging to Dr. H. Woollard.

at the same time tends to give the impression of a greater variation in size in the other parts than really exists. In contrast to the variable scapha and helix, the parts composing the pars articularis (crus helicis, superior and inferior fossae, and the principal fold) exhibit about the same form and relations in each figure.

The principal fold is a little more variable in size than the other parts of the attachment mechanism, although its relations are essentially the same in each case. Where it is particularly well marked it comes to the level of and fuses with the rim of the concha (anthelix), as usually occurs in man. It was this relation which led to the term '*crus inferius anthelicis*.' Where it is not so well developed it does not reach the conchal brim and is thus not continuous with the anthelix. Certainly, in most Primates one cannot properly speak of it as a crus of the anthelix. Since the figures show the chief points that I wish to bring out, it will not be necessary to describe them here individually. It may, however, be well to call attention to the *Tarsius* specimen<sup>3</sup> as the most discordant one in the series. One of its peculiarities is the extraordinary development of what appears to be the principal fold. Instead of constituting a simple strengthening ridge, the fold projects from the conchal floor as a free flap, somewhat of the nature of a similar structure seen in certain bats.

In view of the above observations, we can briefly analyze the form of the primate auricle somewhat as follows: The auricle consists of a primary part (concha), a rigid, shell-like support which is relatively constant in form, and a secondary part (scapha-helix-lobule) which flares from the caudal rim (anthelix) of the concha and is exceedingly variable both in size and form.

The concha in turn may be subdivided into a lower half (cavum conchae), which serves as an approach to the meatus and at the same time provides a closure mechanism by the specialization of its antero-inferior walls, and an upper half

<sup>3</sup> For the privilege of studying this valuable specimen I am indebted to Dr. H. Woollard, of University College, London.



(pars articularis), which constitutes an attachment base from which the auricle as a whole is suspended.

The pars articularis comprises several elements, all of which contribute to its effectiveness. *a)* The superior and inferior articular eminences offer jointly an attachment surface which comes in direct contact with the skull. These eminences are separated by a groove or strengthening fold which in a lateral view projects as the plica principalis, separating the two fossae that correspond to the eminences. *b)* The spinous process (spina) offers a point for ligament and muscle insertion. *c)* The crus helcis forms the anterolateral rim of the pars articularis, its upper end supporting and merging into the helix. Below, it marks the transition of the pars articularis into the lower part of the concha. Including, as we thus do, the pars articularis in the concha, the so-called superior crus of the anthelix becomes simply the upper end of the anthelix, or, in other words, the superior rim of the concha.

Resumen por el autor, Ferdinand C. Lee.

Sobre los vasos linfáticos de la pared de la aorta torácica del gato.

En vista de los escasos conocimientos sobre la irrigación linfática de las paredes de las arterias, el autor publica sus observaciones sobre un ejemplar que indica que en la aorta del gato existe un plexo extenso de capilares linfáticos situado entre las tunicas media y adventicia, mientras que más superficialmente, en la última, existe un plexo de grandes vasos linfáticos los cual es a veces se anastomosan con el plexo situado más profundamente. El ejemplar descrito es un gato en el cual se llevó a cabo la ligadura intratorácica del conducto torácico. Tres semanas después de la operación el animal fué sometido a la acción del éter, inyectando los linfáticos del mesenterio con una solución acuosa saturada de azul de Berlin. La masa de inyección penetró en el torrente circulatorio a través de una conexión linfático-venosa e incidentalmente llenó algunos de los linfáticos de la pared de la aorta.

Translation by José F. Nonidez  
Cornell Medical College, New York

## ON THE LYMPHATIC VESSELS IN THE WALL OF THE THORACIC AORTA OF THE CAT

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### TWO FIGURES

During the course of an investigation involving the intra-thoracic ligation of the thoracic duct of the cat (4), a specimen was obtained which showed to some extent the distribution of lymphatic vessels in the wall of the thoracic aorta. Since the lymphatics of blood-vessels are as yet imperfectly understood, it was considered that the specimen justified this brief report.

The specimen was obtained from an adult male cat whose thoracic duct was ligated according to a method previously described. The animal made an uneventful recovery from the operation, gained slightly in weight, and twenty-one days following the ligation was sacrificed in the following manner. While under the influence of ether, the abdomen was opened and the mesenteric lymphatic vessels were injected with a saturated aqueous solution of Berlin blue, using an ordinary hypodermic syringe with a needle of 28 G. After about 20 cc. of the suspension had been injected, the animal was sacrificed. It was immediately seen that the thoracic duct had been securely ligated, that a lymphaticovenous connection had become established between the thoracic duct and the ninth left intercostal vein, and that a plexus of vessels containing the injection mass existed in the wall of the aorta. The entire trunk of the animal was fixed in 10 per cent formalin, and a few days later a block of tissue containing the lower half of the thoracic aorta and the surrounding tissues was dissected away, dehydrated in alcohol, and cleared according to the Spalteholz method.

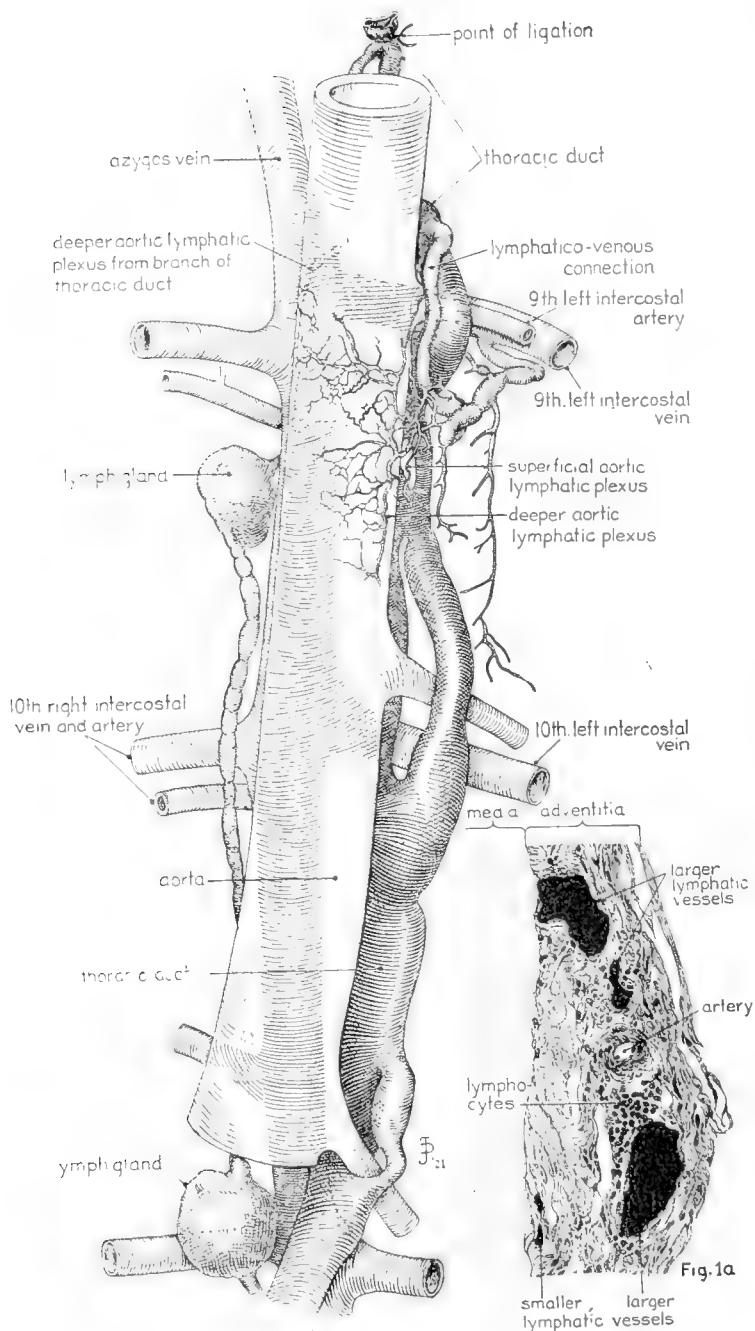


Fig. 1

Figure 1 is a drawing of this specimen as viewed anteriorly. It shows the thoracic duct passing along the thoracic aorta and being pierced, as it were, by successive intercostal arteries. Below the point of ligation the thoracic duct suffers a limited plexiform change, and then, immediately above the ninth left intercostal artery, it gives off a vessel which courses inferiorly and after making several turns goes superiorly and empties into the ninth left intercostal vein. From this lymphaticovenous connection, which possesses numerous valves, vessels come off to form a more superficial and a deeper plexus; the superficial portion in the periadventitial tissue has only a few branches (not striped in the drawing) which communicate at one point with the deeper aortic plexus lying well within the adventitia of that vessel. The lymphaticovenous channel also gives off several branches which course inferiorly in the loose periaortic tissue. The structure of interest is the deeper aortic plexus, which lies definitely in the adventitia; the depth to which it pierces the wall of the aorta is shown in figure 1a. The larger vessels of this plexus have a few valves and soon break up into a rich capillary plexus which extends well over to the right side of the aorta. This capillary plexus lies at the junction of the adventitia and media of that vessel; no branches were seen to pass into the media.

The thoracic duct also gives off a branch, on the right side, posterior to the aorta (fig. 2). This branch courses inferiorly and just before reaching a lymph gland gives off several branches, one of which continues to go inferiorly, while the other branches form a small plexus of capillary vessels, situated also at the junction of the adventitia and media, and extending in a semicircular shape superiorly until it communicates with the thoracic duct. The branch of the thoracic duct leading to the lymph gland is double for a part of its lower course and is abundantly provided with valves. The fine capillary plexus arising in part from this vessel resembles the capillary plexus from the lymphaticovenous connection both in shape and location. These two plexuses approach each other closely at one point, and although the injection shows no continuity between the two, nevertheless a

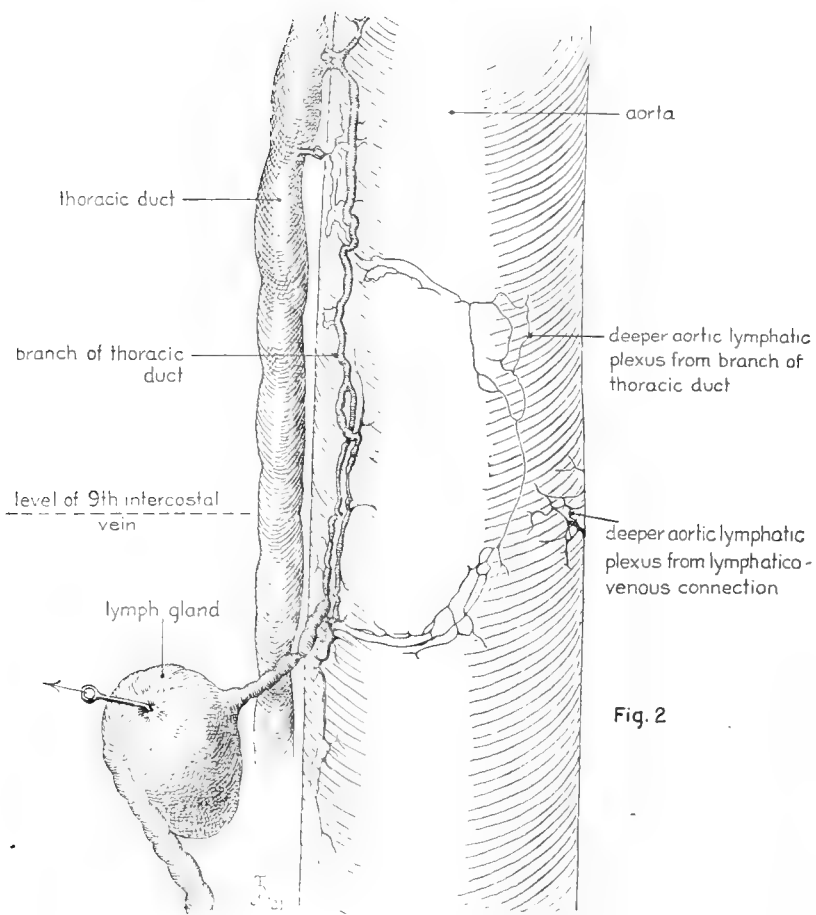


Fig. 2

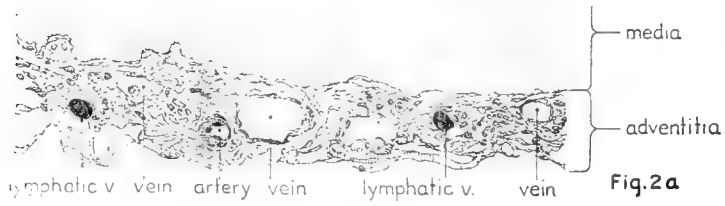


Fig. 2a

small uninjected capillary, discernible with great difficulty, seems to connect one plexus with the other. Along the deeper aortic plexus arising from a branch of the thoracic duct, as shown in figure 2, other vessels, uninjected and paler than the surrounding medium, are seen to course parallel with the lymphatic vessels. These pale uninjected vessels are vasa vasorum and are shown in cross-section in figure 2a.

#### DISCUSSION

Very little is known about the lymphatic vessels in the walls of vessels. Stroganow (7) undoubtedly was dealing with tissue spaces and not true lymphatic vessels in the work which he reported in 1876. Hoggan and Hoggan (3) in 1883, using the silver-nitrate and gold-chloride method in staining the vascular endothelium of the horse, concluded that no lymphatics exist on the inner side of vessel musculature, whether this musculature be in arteries, veins, or large lymphatic trunks, and that only in those large veins or lymphatics in which the muscular coat is absent is there a plexus of lymphatics immediately underneath the lining endothelium.

In 1897 Schiefferdecker (6), using silver lactate, prepared specimens from the pig's aorta that showed a lymphatic plexus directly under the endothelium. Thus he supplemented the work of Hoggan and Hoggan, who found a subendothelial lymphatic plexus only in those vessels in which the muscular coat was absent.

Delamere (2) in 1904, in his classical treatise on the lymphatics stated that the "existence of arterial lymphatics, however probable, is still a mooted point." Bartels (1) in 1909, in his well-known work on the lymphatic system, did not give any new facts concerning the arterial lymphatics.

It is thus obvious that any light which may be thrown on the subject of arterial lymphatics must be of value. It is at once recognized that the specimen here described was obtained from an animal which was subjected to certain operative procedures to its thoracic duct, and from that point of view does not represent a specimen from a strictly normal animal. However, it does give an idea of the general distribution and arrangement

of arterial lymphatics. It shows that the lymphatics to the aorta form not only a superficial, less evident plexus situated in the peripheral portion of the adventitia, but that deeper in the vessel wall, at the junction of the adventitia and media, an extensive capillary lymphatic plexus exists. The larger branches of this deeper lymphatic plexus run parallel with the vasa vasorum of the bloodstream, thus giving the familiar vascular triad of artery, vein, and lymphatic. It also demonstrates how the smallest injected lymphatic capillary is eventually connected with the thoracic duct, without the interposition of a lymph gland (cf. Bartels (1) ).

The determination of the exact character of the plexus arising from the lymphaticovenous connection proper is of importance. Whether this vessel that connects the thoracic duct with the ninth intercostal vein is a lymph vessel or a vein is difficult to decide. Yet the fact that this connecting vessel is tortuous, has so many valves, and gives off vessels which have valves makes one inclined to call it a lymph vessel. And besides it is difficult to consider it a vein because it joins the thoracic duct almost at right angles, whereas ordinarily the typical entrance of a lymph vessel into a vein (e.g., the entrance of the thoracic duct into the junction of the subclavian and internal jugular veins), is such that the lymph vessel makes an acute angle with the vein, and the direction of the current in these two vessels is essentially the same. This arrangement of course facilitates the aspiration of the lymph into the vein. But the direction of the current in the lymphaticovenous connection is opposite to that in the thoracic duct; it thus becomes increasingly difficult to look upon this connecting vessel as a vein. On the other hand, the connecting vessel joins the ninth intercostal vein in such a way that the direction of their respective currents is about the same. The presence of numerous valves in this connecting vessel argues strongly against its interpretation as a vein, because veins of such a small caliber rarely have valves (Schäfer (5)), and if they do have valves, these would probably not be as numerous as the ones in the lymphaticovenous connection.



Granted that the lymphaticovenous connection is essentially a lymph-vessel, then the plexus of vessels which comes from this connection must also be considered lymphatic in character. But, although there are indications of valves in the larger branches of the plexus, nevertheless this entire plexus gives the impression of being venous in character.

As much as one may doubt the lymphatic nature of the plexus communicating with the lymphaticovenous connection, one is all the more sure of the true lymphatic character of the small plexus of vessels in the posterior portion of the aorta. This plexus of vessels communicates only with definite lymphatic trunks, and is therefore definitely lymphatic in character. The finer divisions of this plexus resemble in general appearance and lie at the same depth as the finer vessels of that plexus derived from the lymphaticovenous connection. Unfortunately, no injected vessel was seen to establish the continuity between these two plexuses.

It is a pleasure to thank Mr. J. F. Didusch for the excellent illustrations.

#### SUMMARY

In view of the meager knowledge of the lymphatic supply to the walls of arteries, a specimen is reported here which shows that in the aorta of the cat an extensive plexus of lymphatic capillaries lies at the junction of the media and the adventitia, while more superficially in the adventitia there is a plexus of large lymphatic vessels which occasionally anastomose with the plexus more deeply situated.

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proved to be an accessory pancreas, was located squarely on the free or inferior surface of the gall bladder, and extended backward from the beginning of the neck. It was roughly elliptical in shape. The long axis measured 1.5 cm. and the short axis 1 cm.; it varied from 2 to 4 mm. in thickness. The surface of the gland on the serosa side was irregular and lobulated like a normal pancreas, except that the lobules were small. The mucous membrane of the gall bladder over the gland was not broken, and did not appear different from any other portion, except that there were three pin-point openings. Under the dissecting microscope these appeared to be the openings of ducts. Unfortunately, in the histological sections which were made, it was impossible to follow any of the ducts through to an orifice in the gall bladder, although these ducts were present near the site of the apparent openings. The blood supply to the gland was generous (fig. 1).

Microscopic examination showed the gland to be composed of pancreatic tissue similar to that of the major pancreas (fig. 2). It was normal, except that the acini seemed to be rather small, and there were no well formed islands. There were a few large ducts and several small ones. The acinar cells were closely packed giving a compact appearance to the gland. Small groups of cells apparently island cells, usually not more than six in number, were found at intervals. The careful study of many slides of three accessory pancreatic glands in the dog demonstrated one characteristic common to all, namely, the very small amount of island tissue. In the present case, after a prolonged search, a few definite but very small islands were found. The condition of the acinar tissue in connection with a well developed duct system definitely indicated an active gland, and it was absolutely isolated in every respect from the major pancreas. We examined very carefully around the bit of pancreatic tissue for some indication of a connection between it and the major pancreas, but none was found. Histological sections through the inferior end of the accessory gland failed to disclose any evidence of such a connection. The larger ducts as before noted were toward the gall bladder, into which undoubtedly the secretion was discharged.

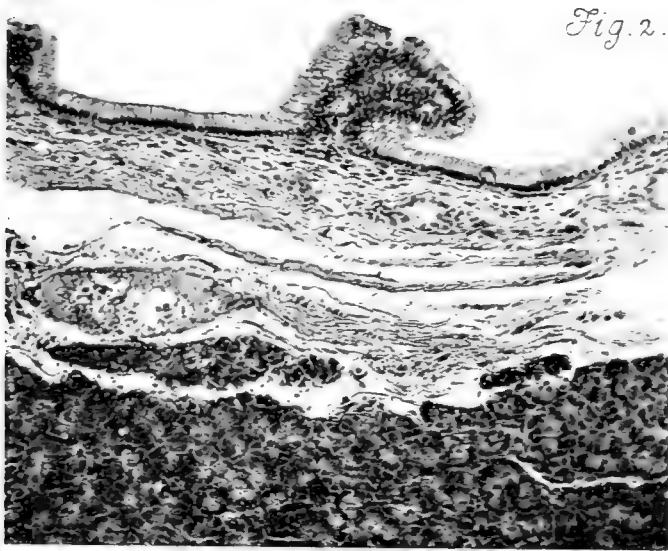
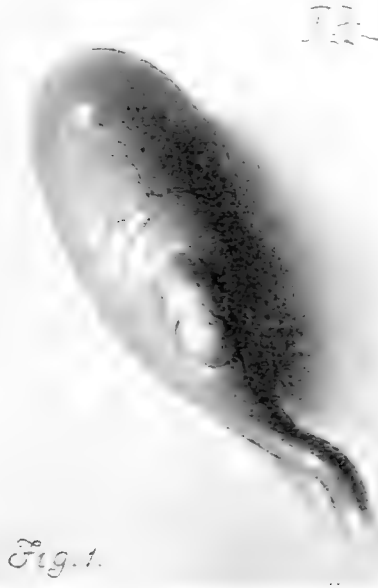


Fig. 1 Drawing of the gall bladder, showing the relative position and size of the accessory pancreas.

Fig. 2 Photomicrograph of section of accessory pancreas and gall-bladder wall. The gland was located just under the serosa. ( $\times 100$ ).

Resumen por el autor, Benjamin T. Nelson.

El número de glomérulos en el riñón del conejo adulto.

El autor ha contado los glomérulos en riñones, en los cuales dichos elementos se habían teñido previamente mediante coloración supravital con verde janus. En uno de ellos ha contado el autor el número total, que resultó ser 163,075. En otros ocho riñones procedentes de cuatro animales, cálculos basados en el peso total de la corteza y en el recuento de un riñón previamente pesado han resultado en cifras que oscilan entre 118,160 y 168,966. Con una sola excepción el número de glomérulos encontrado excede 154,000, y el número medio para todos ellos, incluyendo el riñón contado in toto es 157,180. El número de glomérulos en cada riñón del conejo adulto es por consiguiente unos 160,000, próximamente.

Translation by José F. Nonidez  
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## THE NUMBER OF GLOMERULI IN THE KIDNEY OF THE ADULT RABBIT

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The number of glomeruli in a kidney is a measure of the number of uriniferous tubules, and as such a necessary factor in estimating the relative surface of glomerular and tubular epithelium concerned in the secretion of the urine. It is also an important element in Brodie's computation of the pressure necessary at the glomerular end of the tubule to drive the urine along the tubule at the rate at which it is secreted in diuresis.

The earliest enumeration of the tubules is credited to Eysenhardt (1818), whose article I have, unfortunately, been unable to consult. According to Huschke ('44), Eysenhardt estimated the tubules of the human kidney at 42,000,000—a number which Huschke criticised as probably much too high because of the enumeration of blood vessels as tubules. Through an error Huschke is usually credited by authors as the first to enumerate the glomeruli, but his article in Oken's *Isis*, vol. 21, 1828, to which reference is made by Brodie, Policard, and others, contains no mention of the number, except the remark that they are more numerous, relative to mass, in the young than in the adult. Not until 1844, however, did Huschke discuss the actual number of tubules in the human kidney. In his article entitled "Einge-weidelehre" in Sömmering's *Bau des menschlichen Körpers* he said: "Every kidney lobe contains about 700 kidney lobules and each lobule about 200 cortical canals. If the kidney has 15 lobes that would give it 10,500 lobules and 2,100,000 cortical canals."

In his classical work on the kidney published in 1865, Schweigger-Seidel ('65) describes his method of determining the number of glomeruli in the kidney of the pig and discusses the results.

After carefully separating the cortical substance from the rest of the renal substance, he found, in a kidney weighing 120.5 grams, 102 grams of cortex. Small weighed portions of this cortex were teased and macerated in hydrochloric acid to permit the separation of the glomeruli for counting. In a total weight of 15.5 ggm. he found 720 glomeruli, and calculated the total content of the kidney as 473,200 glomeruli.

Peter's ('09) estimate of the number of tubules in the cat is based on the mode of branching of the ducts. He says: "Bei der Katze ergeben sich stets 4 initiale Aeste und meist 7 zentrale Teilungen; auf einen ins Becken mündenden Gang entfallen damit (2<sup>3</sup>). 4 Kanälchen, d.h. 1024; nehmen wir die Zahl der Sammelröhren 1. Ordnung wie beim Hund auf 200-300 an, so ergäbe dies für jede Niere 200,000-300,000 Harnkanälchen." The number of tubules arising from a duct of the first order, 1024, was incorrectly quoted by Policard ('08) as Peter's estimate for the whole kidney.

Miller and Carlton in 1895 made an enumeration of the glomeruli in the cat kidney, based on a previous determination of the average volume of the cortex. They cut sections in series at a thickness of 0.10 mm. of a kidney injected with Prussian-blue gelatin. The outline of the section traced on paper and measured with a planimeter gave the area. They found that 50 per cent to 83 per cent of the glomeruli represented the actual number, the rest representing duplicate counts of glomeruli appearing in more than one section. They computed the content of an average volume kidney of 12.9 cc. containing 9.03 cc. of cortex in one estimation as from 9,183.49 to 15,325.13 glomeruli. Another estimation gave them from 13,288 to 22,220 glomeruli. The mean of these estimates is 15,664.

Brodie ('14) in 1914, with the assistance of Miss M. G. Thackrah, estimated the glomerular content in two dog kidneys, using a method similar to Miller and Carlton's. Brodie and Thackrah, however, cut a complete series of sections, 8  $\mu$  thick, of previously weighed pieces of cortex. In these sections the total number of glomeruli was counted and divided by the number of sections in which on the average a single glomerulus would



appear. In this way a ratio of number of glomeruli to weight was obtained from which the whole content of the cortex could easily be computed. The first dog weighed 11 kgrm., its right kidney weighed 34.5 grams, and the total number of glomeruli was 142,000. A kidney of a second dog, weighing a little over 8 kgrm., contained 125,000 glomeruli.

It is difficult to find a basis for comparison of these results because in the case of the cat only have we estimates made by two investigators. In this case the estimate of Peters is more than tenfold that of Miller and Carlton. The method employed by Miller and Carlton, however, involved the actual count of the glomeruli in series, and thus is less open to objection than that of Peters, who used in his computation a factor obtained from study of the dog's kidney.

If we compare in so far as is practicable the results of Miller and Carlton on the cat with those of Brodie on the dog, we find that Brodie's result, though agreeing fairly well with the estimate of Schweigger-Seidel on the pig, involves a content per milligram of kidney approximately three times as great as that of Miller and Carlton. The recent work of Bremer ('16) on the activity of the mesonephros in the embryos of various mammals, as indicated by the number and size of the glomeruli and by the increase in number and size of them in progressive phases of embryonic development, may throw some light on the variations in glomerular content of the kidneys of different species of mammals. Bremer found "that the different embryos can be classed as those which retain a functional wolffian body until the kidney is ready to take up the work of excretion, and those in which the wolffian body disappears early, before the kidney has developed active glomeruli." He finds also in the placenta of the latter group evidences of anatomical specialization for interchange with the maternal circulation, which justify the assumption of an excretory function on the part of the placenta. Thus it appears probable that the development of the kidney may be influenced by the concurrent existence, functional capacity, and history of other excretory mechanisms. Bremer also points out that within each of the classes which he recog-

nizes, "individual animals are provided with a very varying amount of excreting surface, showing presumably varying types of metabolism." The discussion of these interesting correlations must, however, be postponed, pending a confirmation by newer and better methods of the counts of glomeruli made in other mammals, which must in addition be supplemented by a careful quantitative study of the relative total glomerular surfaces—a study which involves a consideration not only of the number, but also of the size and lobulation of glomeruli.

My determinations have been made by a method essentially similar to that employed by Schweigger-Seidel, except that in most cases I counted the glomeruli in a much larger percentage of the cortex and, in two cases counted the glomeruli in the entire cortex of an adult rabbit's kidney. In addition, the glomeruli were stained so that there was no difficulty in seeing them and distinguishing them from tubules, which might be difficult in the unstained kidney used by Schweigger-Seidel.

The method is essentially the same as that employed by Bensley in the enumeration of the islands of Langerhans in the pancreas. Janus green B when injected by way of the blood vessels in the living kidney, has the property of staining the glomerular tufts intensely, and the stain can be easily fixed permanently by means of ammonium molybdate (Merck or Kahlbaum). According to Cowdry ('18), janus blue has the same property, but we have not employed this dye.

The animal is killed by bleeding from the carotid, the chest rapidly opened and a cannula inserted in the arch of the aorta. Through this 0.85 per cent salt solution is injected until the blood is well washed out of the kidney, when it is followed by a 1 in. 10,000 solution of janus green in salt solution. When the kidney is a uniform blue color, it is covered up by the intestines for fifteen to thirty minutes, to permit reduction of the excess dye. When this process is complete the kidney presents a purplish tint on its surface. Then a 5 per cent solution of ammonium molybdate is injected to check further reduction, and the kidney is removed and placed in a jar containing molybdate. In a successful preparation only the glomeruli will be deeply

stained blue, and as Cowdry (*loc. cit.*) remarked, they can be seen and counted even in thick pieces.

For counting it is necessary to separate the cortex from the medulla, except where a total count is to be made. This involves no great difficulty, since the cortex is deeply stained blue from the numerous glomeruli contained in it. To accomplish this, the kidney is cut into ten or twelve thick slices, out of which the medulla is carefully cut with a scalpel. When this is done a certain amount of fluid escapes, so it is important to determine at the same phase of the work the ratio of weight of sample counted to whole cortex. I have found also that pieces left in ammonium molybdate for some time change considerably in weight. As soon as the cortex is separated from the medulla the total weight is obtained, and a piece selected for counting and immediately weighed.

For counting, the method of teasing and compression is employed. The block to be counted is divided into small fragments which are teased apart and then compressed between two slides, or under a thick cover-glass. Counting is not difficult, and I am confident that the errors of counting are few. Doubtless some error is introduced by incomplete staining and by irregular oedema of the parts of the kidney. Another source of error is incomplete separation of medulla from cortex. As a check on the method of estimation, ten estimates and a complete count were made on the left kidney of a female rabbit weighing 1470 grams. The kidney contained by actual count 227,263 glomeruli. The average of the estimates was 212,269—an error of 6.7 per cent; but the error of the individual estimates varied from 19 per cent plus to 27.7 per cent minus. However, the error of six of the ten estimates was less than 9 per cent and of four less than 4 per cent. A plus error may arise through selecting for counting a piece from the surface of the cortex where the ratio of *pars convoluta* to *pars radiata* is high, and conversely a minus error may arise, as the table shows clearly, from incomplete separation of the medulla from cortex. By care in selection of the sample to be counted, the error may be reduced to less than 10 per cent. The results are contained in the subjoined tables.

The results of these counts and estimates are unexpectedly high. The actual content of glomeruli in a rabbit's kidney is tenfold that admitted for the kidney of the cat by Miller and Carlton, and more than one-third of the content found by Schweigger-Seidel in a pig's kidney fifteen times as heavy. Such differences, if true, suggest interesting physiological and anatomical implications, into the discussion of which, however, we cannot enter at present, pending the confirmation by newer and more accurate methods, of the results obtained by other investigators. This work is now in progress.

TABLE 1

NO.	SEX	BODY WEIGHT	KIDNEY WEIGHT <sup>1</sup>	CORTEX WEIGHT	WEIGHT OF SAMPLE	GLOMERULI	TOTAL
			gm.				
1	M	1990	Total count of one kidney				163075
2	M	1665	R 6.09 L 6.56	3.69 3.73	0.21 0.19	9221 8171	161991 160390
3	F	2145	R 8.40 L 8.68	5.38 5.54	0.36 0.42	10460 11733	156020 154566
4	M	2040	R 8.22 L 8.27	5.4 5.6	0.33 0.175	10326 3704	168966 118160
5	M	2000	R 8.51 L 8.24	5.1 4.6	0.165 0.290	5405 10387	166770 164680

<sup>1</sup> The weights of the kidney given in this table have no value except in relation to the counts, because different kidneys acquire different degrees of oedema in the process of staining.

TABLE 2

*Female rabbit, weight 1470 grams*

	Glomeruli
Total weight of kidney 9.98 grams.....	227,263
Total weight of cortex 4.85 grams.....	223,150
Total weight of medulla 5.13 grams.....	4,113
(plus cortex in separation)	

TABLE 2—Continued

*Estimations*

NUMBER	WEIGHT	MEDULLA PLUS OR MINUS	COUNT	ESTIMATE	PER CENT OF ERROR
1	0.160	Not observed	7,175	217,488	4.3 minus
2	0.140	Minus	7,843	270,846	19.1 plus
3	0.250	Plus	10,668	206,959	9.3 minus
4	0.280	Plus	9,482	164,228	27.7 minus
5	0.220	Minus	10,597	233,557	2.7 plus
6	0.250	Minus	11,868	230,439	1.3 plus
7	0.250	Minus	11,700	226,980	0.1 minus
8	0.290	Plus	12,395	207,244	8.8 minus
9	0.270	Plus	9,633	173,008	23.8 minus
10	0.230	Plus	9,081	191,427	15.7 minus
Average of the estimates.....				212,269	6.7 minus

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Resumen por la autora, Mary T. Harman.

Sobre el origen del notocordio en el pollo.

Dos pollos anormales producidos en nuestro laboratorio presentaban notocordios excepcionales. Ambos fueron incubados durante tres días. Uno de ellos fué incubado a una temperatura de 106°F. y el otro a una temperatura de 99° a 100°F. En el primer pollo el notocordio es bifurcado en su extremo anterior, en próximamente un tercio de su longitud. En esta misma región el tubo neural está también bifurcado ventralmente. El intestino no presenta indicio alguno de bifurcación. En el segundo pollo el notocordio en el extremo posterior no solo está en contacto con el tubo neural, sino que se continúa con él. El endodermo del saco vitelino está por completo separado del notocordio. Las condiciones excepcionales descritas constituyen pruebas en favor de la teoría del origen ectodérmico del notocordio.

Translation by José F. Nonidez  
Cornell Medical College, New York

## CONCERNING THE ORIGIN OF THE NOTOCHORD IN THE CHICK<sup>1</sup>

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ONE PLATE (FIVE FIGURES)

The presence of the chorda dorsalis or notochord sometime during the life-history is one of the characteristics which distinguishes the Chordata from the other phyla of the animal kingdom. As to its origin, few other embryonic structures have received more attention from investigators, and yet there exists the greatest diversity of opinion concerning its early embryonic development. All three germ layers have been given as its origin. As early as 1881 Hatschek described the notochord as being folded off from the primitive gut. This is the view held by Hertwig ('05), McMurich ('15), and others. Prentiss and Arey ('17) say that "the head (notochordal) process and mesoderm of higher vertebrates are not clearly of entodermal origin, but are derived from the ectoderm, any union with the entoderm being secondary." Huber ('18) also considers the notochord to be of ectodermal origin, which becomes associated with the entoderm, and then afterward separated from it. King ('03) says that in *Bufo lentiginosus* the anterior part of the notochord is entirely mesodermal in origin, and that in the posterior part it is mostly derived from mesoderm, but that there is added to it a layer of entoderm from the archenteron.

These differences of opinion are not entirely due to the fact that the investigators have been working with different vertebrates, for Foster and Balfour ('83) say that in the chick the notochord in most instances arises from the anterior end of the

<sup>1</sup> Contribution from the Zoological Laboratory, Kansas State Agricultural College, no. 57.

primitive streak and remains attached to the 'hypoblast' (entoderm), but in other cases the notochord appears to become differentiated in the already separated layer of mesoderm. Prentiss and Arey ('17) say that the notochordal plate in birds is mesodermal in origin and later becomes fused with the entoderm. However, they state that the mesodermal plate which goes to form the notochord arises from the thickened ectoderm of the primitive streak.

These great differences of the opinions of able investigators have led the writer to record some abnormal conditions in the notochord of some teratological specimens of the chick produced in our laboratory (Harman, '18; Alsop, '19). It is not the purpose of the writer to give an extended discussion of the vast amount of literature on the origin of the notochord which must of necessity include the origin of the germ layers, but rather to present the conditions as found in these specimens and discuss to some extent the possible bearing upon the theories of the origin of the notochord.

The illustrations are taken from two of the specimens referred to above. Both chicks were incubated for three days. One was incubated at a temperature of about 106°F., and in addition to a very abnormal central nervous system has a bifurcate notochord. The double condition is in the anterior end and extends caudad to the region of the origin of the vitelline veins. Transverse sections of this specimen are represented in figures 1, 2, and 3. The other chick was incubated at a temperature of between 99°F. and 100°F. The abnormal condition of the notochord is shown in figures 4 and 5.

Figure 1 is a section through the chick about 50 $\mu$  from the extreme anterior end of the notochord. The notochord, marked *nc* in the figure, is rather small, cylindrical in shape, and instead of being in a median line ventral to the hind-brain, marked *nt* in the drawing, it is in two parts or cylinders located a little to either side of the midventral line. The hind-brain in this region encloses two cavities, a larger dorsal cavity and a very small ventral one. The small cavity is slightly forked ventrally. The respective horns are in a direct line with the noto-



chord of that side. The pharynx, marked *g* in this section, has no indication of the double condition dorsally.

The notochord is double for a distance of about  $1\frac{1}{4}$  mm. or through the heart region, a distance of about one-third of its entire length. Figure 3 is a drawing of a section at the point of the convergence of the two horns of the notochord. Figure 2 is a drawing of a section about midway between the sections represented in figures 1 and 3. It will be noted that in figure 3 the notochord is large compared with the double notochord represented in the other two figures. It also has a slight indication of its double condition. The neural tube here also is somewhat double ventrally. Posterior to this region, both the notochord and neural tube are single and appear to be normal for this stage of development. Anterior to this region, the two parts of the notochord gradually diverge and the respective horns become smaller to the region represented in figure 2. Then they come a little closer together, but remain about the same size, as is shown in figure 1. The two cavities of the central nervous system are found only in three sections, but the bifurcate condition of the ventral side of the central nervous system is very distinct in the entire region of the double notochord.

Figures 4 and 5 are drawings of sections through the posterior third of a three-day chick incubated at a temperature between 99°F. and 100°F. The section represented by figure 4 is about  $\frac{1}{3}$  mm. anterior to the other one. The neural tube in this region is not quite normal, but its abnormality is more striking a few sections anterior where they are two and three central canals as previously described (Harman, '18; Alsop, '19). As shown in figure 4, the notochord seems to be directly continuous with the neural tube, although the two are almost separated. The entoderm, *en*, of the yolk sac lies close to but entirely separated from the notochord. Posteriorly, as shown in figure 5, the continuity of the notochord with the neural tube is more conspicuous. In fact, the constriction between the notochord and the neural tube is not very deep, and an examination of the cells with the high power of the microscope shows that they are of the same character in both of the structures. The entoderm of the yolk sac is in contact with the notochord, but is distinct from it.

In the case of the first chick, two hypotheses may be considered: either the notochord became bifurcate at the time of its formation or after it was formed. If the former, the factor or factors which by their influence caused the doubling would have affected the structure from which the notochord originated and we should expect an indication, at least, of a double condition of this structure; if the latter, we must suppose the doubling to be accompanied by a growth in its diameter, since each of the paired notochords is of nearly normal size. It is well known that increase in diameter is not normally noticeable in the chick notochord, as the chief difference between that of a one-day chick and that of a three-day chick is in its length. It is evident that increase in length could not produce bifurcation. Therefore, it is hardly probable that bifurcation occurred after the notochord was formed, and we can return to the assumption that the notochord became bifurcate at the time of its formation.

Surrounding the notochord is mesoderm, ventral to it is the gut which is entodermal in origin, and dorsal to it is the neural tube which is ectodermal in origin. If the notochord came from the surrounding mesoderm, it probably developed in situ, since there is no indication of a doubling of the mesodermal structures. The writer sees no condition in favor of this hypothesis and on the other hand no direct evidence against it. If it has arisen in situ it is unique among compact structures of embryonic development which usually arise from a region of unequal proliferation of cells which finally results in a folding or in the formation of a thickened plate. Since there is no evidence of an unequal proliferation of mesodermal cells, the indirect evidence is rather against the theory of the mesodermal origin of the notochord.

If we assume that the notochord is entodermal in origin, we will have to accept that it came from the gut or from the yolk sac before the gut was formed, for these are the only structures of this region of entodermal origin. There is no indication of a doubling of the gut; therefore, from our hypothesis the notochord cannot be entodermal in origin. Then it must be ectodermal, since, as we have shown, it is hardly probable that it is either mesodermal or endodermal. Let us examine the evidence for the hypothesis of its ectodermal origin.

If it were ectodermal in origin, it must have come from the ventral surface of the neural tube. As we have shown previously, the neural tube in the region of the double notochord is double ventrally and where it is single the neural tube is also single. Furthermore, in a normal chick the notochord lies ventral to the median line of the neural tube and the single *canalis centralis* is in direct line with the notochord. In this specimen the *canalis centralis* is forked ventrally. If the respective forks of the *canalis centralis* should be considered as being in the ventral portion of a complete neural tube, then the notochord of the respective sides would have a normal position with reference to the neural tube.

It would seem, then, either that some factor or factors affected the notochord and the neural tube, at the same time causing both of them to bifurcate at least partially, or that they caused the formation of two neural grooves instead of one and that the notochord arose from the ventral surface of the neural tube. This seems all the more probable since both structures are double in the same place and since they are the only structures of this region showing this condition. Therefore, the facts indicate that the notochord became forked at the time it was formed and that it came from the ectoderm.

The condition in the second chick gives added evidence that the notochord has arisen from the ventral part of the neural tube or at least from the ectoderm from which the neural tube is formed. As was given above in figure 4, the neural tube is continuous with the notochord, while in figure 5 not only are they continuous, but it is impossible to tell where neural tube stops and notochord begins. Moreover, the entoderm of the yolk sac is completely separated from the notochord. It seems, then, that the notochord is ectodermal in origin.

To summarize, then, the bifurcate condition of the notochord and a similar condition in the ventral portion of the neural tube in the case of the first described, together with the absence of evidence of a double condition in any other structure in this region, is evidence in favor of the theory that the notochord in the chick is ectodermal in origin. In the case of the second chick, the continuity of the cells of the neural tube with those of the

notochord as well as the complete separation of the entoderm of the yolk sac from the notochord is added evidence for the theory of the ectodermal origin of the notochord.

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#### PLATE I

##### EXPLANATION OF FIGURES

All figures were enlarged about 135 diameters and then reduced one-half.

Figures 1, 2, and 3 are sections through a three-day chick incubated at 106° F. Figures 4 and 5 are sections through a three-day chick incubated at 99° F. to 100° F.

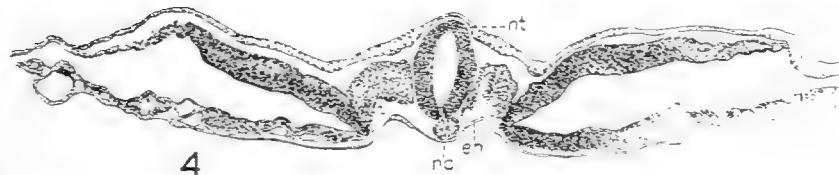
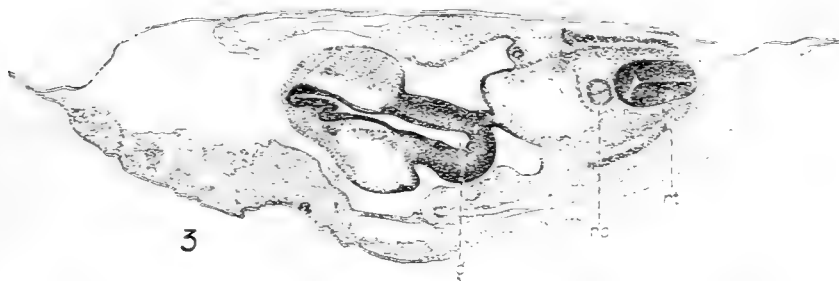
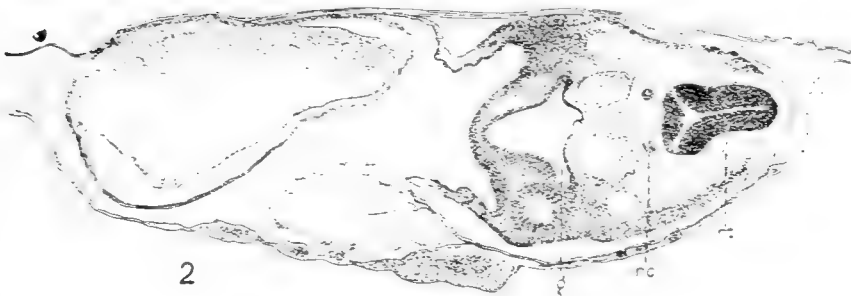
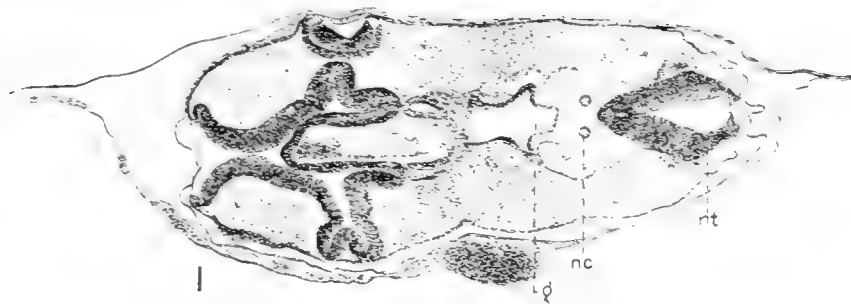
1 Section through the anterior portion of the notochord. *nc*, notochord; *g*, gut; *nt*, neural tube.

2 Section through the middle of the heart. *nc*, notochord; *g*, gut; *nt*, neural tube.

3 Section through the origin of the vitelline veins. *nc*, notochord; *g*, gut; *nt*, neural tube.

4 Section through the chick about one-third of its length from the posterior end. *nt*, neural tube; *nc*, notochord, *en*, entoderm of yolk sac.

5 Section through the chick about  $\frac{1}{2}$  mm. posterior to the section represented in figure 4.



Resumen por el autor, Walter Hughson.

La estimulación eléctrica de los nervios cutáneos. Un método de enseñanza.

La estimulación eléctrica de los troncos nerviosos cutáneos ha sido empleada por el autor como un método de enseñanza de los estudiantes de Anatomía macroscópica. Trotter y Davies han demostrado la exactitud de este método y su uso ha sido valioso por suministrar al estudiante conocimientos prácticos de la distribución final y variaciones de estos nervios.

Translation by José F. Nonidez  
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## ELECTRICAL STIMULATION OF CUTANEOUS NERVES: A TEACHING METHOD

WALTER HUGHSON

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TWO FIGURES

The usual method of teaching the distribution of cutaneous nerves to students in gross anatomy must be regarded in a general way as unsatisfactory. Careful dissections of individual nerve trunks do little more than indicate roughly the region supplied, while text-books with their variegated illustrations leave in the mind of the student a confused idea of some association between cutaneous sensibility and one or more of the different colors used. Such an interesting and important detail as variation in the ultimate distribution of a given nerve is almost impossible of demonstration with our present methods. In short, the student's whole conception is so vague that after having completed his course in anatomy he promptly loses any detailed knowledge he may have gained by his study of text-books and dissections.

Trotter and Davies,<sup>1</sup> in their work on the innervation of the skin, attempted to establish definite anatomic relations for several cutaneous nerves. Variations in the course of the nerves were so great, however, that this was found impossible. Exposure of the nerve trunk for section which formed part of their experiment often involved a skin incision out of all proportion to the size of the procedure. They therefore devised a method of localization which consisted simply of stimulating the nerve-trunk through the skin with a weak faradic current by a finely pointed unipolar electrode. The sensation of tingling corresponded exactly to the peripheral distribution of the nerve.

<sup>1</sup> Trotter, W., and Davies, H. M., *Jour. of Phys.*, 1909, vol. 38, p. 134.

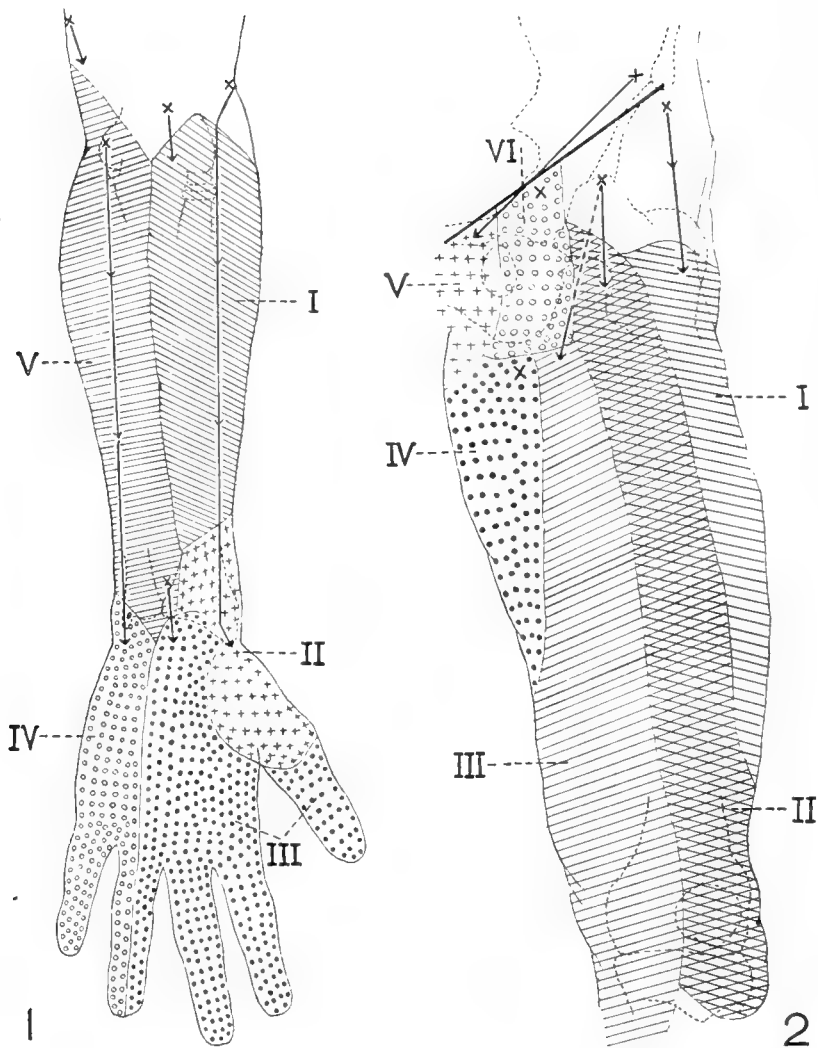


Fig. 1 Volar surface left forearm. *I*, Lateral antibrachial cutaneous; *II*, cutaneous distribution of radial; *III*, of median, and *IV*, of ulnar; *V*, median antibrachial cutaneous.

Fig. 2 Anterior aspect of left thigh. *I*, Lateral femoral cutaneous; *II*, intermediate; and *III*, medial cutaneous branches of femoral; *IV*, cutaneous branch of obturator; *V*, of ilio-inguinal, and *VI*, of genito-femoral.



Application of the stigmatic electrode a millimeter or more to either side of the nerve-trunk failed to elicit any sensation; the accuracy of the method was further demonstrated at operation by finding the nerve immediately beneath the point of localization on the skin. After section, the area of anesthesia corresponded exactly to the distribution determined by stimulation.

The method was used in an elective course in applied anatomy to show the distribution of the cutaneous nerves throughout practically the entire body. A small induction coil (Harvard Instrument Company) was used with a single dry cell. A current considerably weaker than that necessary to cause muscular contraction was found to be sufficient to stimulate the sensory nerves. The indifferent electrode was placed at any convenient point on the body and the stimulating electrode applied to the skin to locate the nerve trunk as it emerged from the deep fascia. The general position of the nerve had previously been determined by the student by dissection. The distinctness of the response to stimulation proved to be very striking and the areas could be mapped out after a small amount of practice with great accuracy.

Figures 1 and 2 represent the type of diagram which the individual student can make. The entire procedure can be carried on without assistance. The nerve is first picked up at the point where it pierces the fascia and can then be followed in all its branches. In the figures, X marks the point of stimulation and the arrow the area of distribution indicated by shading. In figure 1 the arrow shows that stimulation of the radial and ulnar cutaneous nerves along their entire course from the elbow will give the typical sensory distribution. The cutaneous branches of the median nerve, however, can be stimulated on the volar surface of the forearm only at the wrist. The lateral and medial cutaneous nerves of the forearm can best be stimulated above the elbow. Figure 2 represents a fairly typical diagram of the cutaneous innervation of the front of the thigh. The intermediate branch of the femoral becomes superficial higher up than the medial branch, as is indicated by dotted lines, but the whole distribution is clearly shown. The cutaneous

branch of the obturator is surprisingly accessible. Of some interest is the fact that in this individual the obturator of the opposite side had an area of distribution considerably below the knee. The ilio-inguinal can be found very readily above the inguinal ligament. The whole body with the possible exception of the intercostal nerves can be charted in exactly the same manner. Stimulation of the cutaneous branches of the trigeminal, the branches of the cervical plexus, etc., all give the sharp outline indicated in the figures.

This method then gives the student an actual physiological proof of the distribution of his own cutaneous nerves and fixes this distribution firmly in his mind as dissections and pictures can never do. By comparison with other charts he learns that there is a great variation between different individuals and even between opposite sides of the same individual. And finally the simplicity of the apparatus and method makes it entirely practical for use in large classes.



Resumen por el autor, I. M. Thompson.

Anomalía del nervio medio y del músculo flexor sublime de los dedos.

El autor describe una anomalía del nervio medio del antebrazo, el cual estaba enterrado en el vientre radial del flexor sublime de los dedos, en la mayor parte de su curso en el antebrazo. Dicho músculo era también anormal, presentando separados los orígenes radial y húmero-ulnar, y este último estaba subdividido de nuevo inmediatamente debajo del codo en un vientre lateral pequeño y un vientre medio grande. La disposición de los tendones de este músculo en la muñeca era también excepcional. El autor presenta una explicación embriológica de la anomalía descrita, indicando también su importancia práctica.

Translation by José F. Nonidez  
Cornell Medical College, New York

## ANOMALY OF MEDIAN NERVE AND FLEXOR DIGITORUM SUBLIMIS MUSCLE

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### ONE FIGURE

The following anomaly was observed in the dissecting-room at McGill University during the session of 1921-22. As I have not succeeded in finding a description of the condition in the usual records, it would appear to be uncommon, and its importance from a practical point of view makes it the more worthy of being recorded.

The subject was a female, aged eighty-six, with attenuated musculature. The median nerve of the right-arm descended into the forearm between the two heads of the pronator teres in the usual manner. Then it passed deep to the radial origin of the flexor digitorum sublimis, the latter consisting of a slender belly which was not united to the humero-ulnar origin. About 35 mm. distal to this point (i.e., about 130 mm. distal to the medial epicondyle of the humerus) the nerve sank into the substance of the radial belly, and remained completely embedded therein down to a point about 25 mm. above the wrist-joint. Here the tendon of this belly divided into two, a medial and a lateral; and the nerve appeared between them, lying anterior to the lateral tendon and between the medial tendon and that of the flexor carpi radialis. The subsequent course and distribution of the nerve presented no unusual feature.

The following associated conditions may be mentioned. As indicated, the flexor digitorum sublimis was abnormal. In the first place, the radial and humero-ulnar origins remained entirely separate. Just below the elbow the humero-ulnar origin divided into a large medial belly and a smaller lateral belly.

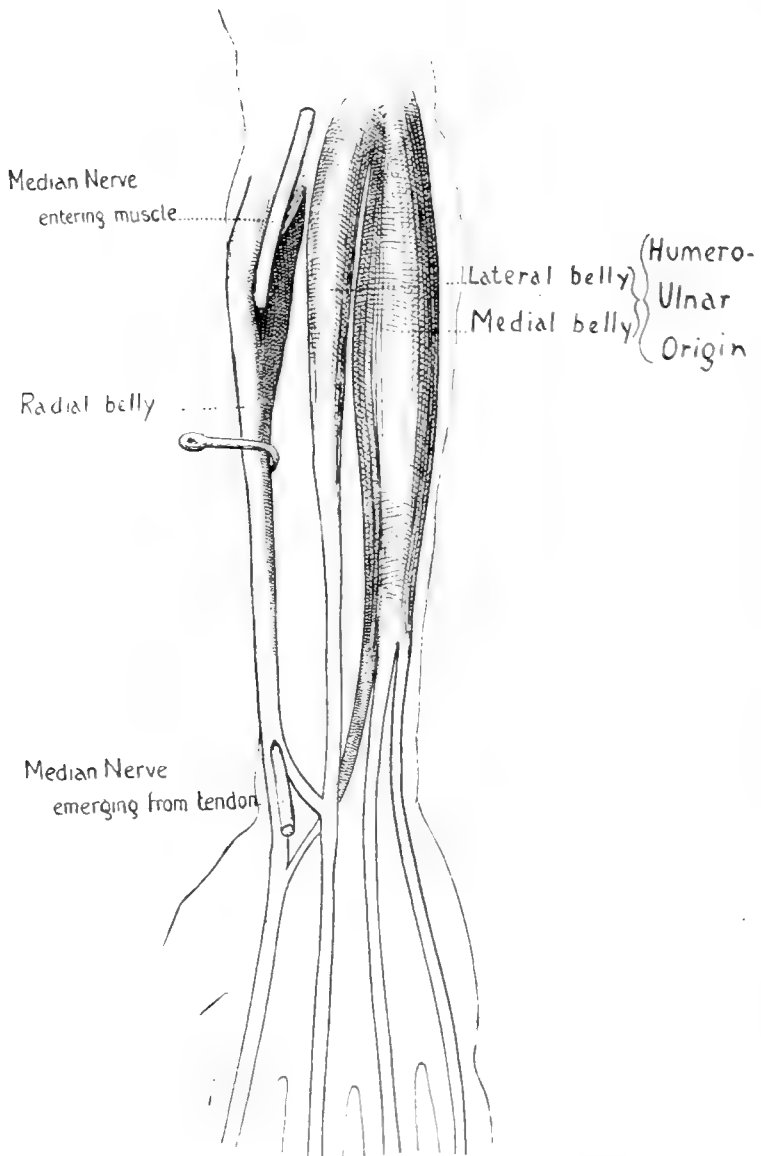


Fig. 1 Right forearm and hand, volar aspect, showing anomalous median nerve and flexor digitorum sublimis muscle. The upper part of the radial belly has been retracted laterally and twisted, exposing its deep or dorsal aspect, with the median nerve entering the muscle. For further description see text.

About 60 mm. above the wrist-joint the medial belly was continued into two tendons, a medial and a lateral, with the addition of a small fleshy lateral slip. The smaller medial tendon went to the little finger, whilst the larger lateral tendon terminated in the ring-finger. About the middle of the forearm the lateral humero-ulnar belly was continued into a tendon which went to the middle finger, being joined at the level of the wrist-joint by the medial tendon of the radial belly.

The slender separate radial belly, in which the median nerve was embedded, arose by fleshy fibers from the lower end of the oblique line of the radius. About the middle of the forearm it gave rise to two tendons, a medial and a lateral, which were closely bound together (enclosing between them the median nerve) down to a point about 25 mm. above the wrist-joint, where they separated. The medial tendon joined the tendon of the lateral humero-ulnar belly to form the tendon of the middle finger. The lateral tendon went to the index finger, being joined about 20 mm. below the wrist-joint by the tendon of the small fleshy slip noted above as arising from the lower end of the lateral aspect of the medial humero-ulnar belly, this fleshy slip having become tendinous where it crossed deep to the tendon of the middle finger, at the level of the wrist-joint. The ultimate insertions of these tendons in the fingers presented no unusual feature.

The palmaris longus was absent on both sides.

On the right side the ulnar artery arose as a collateral branch of the brachial artery opposite the upper end of the insertion of the coracobrachialis. It was small, and passed superficial to the common flexor group of muscles. The radial artery was the direct continuation of the brachial artery, was larger than usual, and gave off the common interosseous artery.

On the left side there were no anomalies except the absence of the palmaris longus.

Normally, the median nerve is closely adherent to the deep surface of the flexor digitorum sublimis, to which it is bound by relatively dense connective tissue. This fact is not stated in the majority of the text-books of anatomy, but is easily

verified by dissection. The present anomaly appears to be a further stage of this normal condition, the nerve not merely adhering to the deep surface of the muscle, but actually being embedded within its substance.

W. H. Lewis (The development of the arm in man; *Am. Jour. Anat.*, vol. 1, 1902, p. 145) describes the median nerve in a human embryo of 9 mm. as extending to the distal end of the humerus, while the distal part of the arm is filled with primitive mesenchymal tissue which later differentiates into muscle and intermuscular tissue.

In an embryo of 10.5 mm. the same investigator describes the flexor muscle mass of the forearm as consisting of a superficial layer and a deep layer, separable with considerable difficulty, the median nerve lying between them.

In the 9-mm. embryo the median nerve has reached the distal end of the humerus, while the premuscle tissue of the upper arm shows no sign of differentiation. Assuming that what occurs in the arm occurs also in the forearm, we may therefore conclude that between these two stages described by Lewis the median nerve grows down into the undifferentiated mesenchyma of the forearm, which thereafter develops two planes of histogenic demarkation, a superficial plane, anterior to the median nerve, along which the superficial muscle layer is differentiated from the layer of connective tissue which intervenes between the superficial and the deep muscles, and a deep plane, posterior to the median nerve, along which the deep muscle layer is differentiated from the same layer of connective tissue. The median nerve thus comes to lie within the layer of connective tissue, between the two planes of demarkation, but nearer the superficial plane, in which region the connective tissue increases in density, thus binding the nerve to the deep surface of the muscle.

The present anomaly has probably been occasioned by the superficial plane of demarkation passing posterior to the median nerve, which was thus included within the superficial layer of muscle tissue from which the flexor digitorum sublimis was later differentiated.



This anomaly would appear to be of some clinical importance, though no information is available in the present case as to the production of symptoms by such an anatomical condition. In this connection it may be noted that normally certain nerves lie embedded in muscle for a portion of their course, as, for example, the deep branch of the radial nerve, which lies embedded in the supinator.

In those cases which call for operative treatment of the flexor tendons or of the median nerve in the forearm, and in which difficulty is experienced in locating the nerve or its ends, if it has been severed, the possibility of the presence of such an anomaly as that described would appear to be worth bearing in mind.

In the present instance, the nerve, as noted, entered the muscle about 130 mm. distal to the medial epicondyle of the humerus, and emerged from the tendon about 25 mm., or one inch, above the wrist-joint. In the living this would correspond approximately to a point about 25 mm., or one inch, above the proximal skin crease at the wrist.

Resumen por el autor, William H. T. Baillie.

Un caso de ausencia unilateral del riñón, uréter y parte distal del tubo uterino del conejo.

El autor describe una caso, hallado en una coneja, de ausencia del riñón y uréter izquierdos, parte del tubo uterino izquierdo y útero del mismo lado. Teniendo en cuenta las condiciones halladas durante el desarrollo embrionario el origen de la anomalía debe haber tenido lugar próximamente en un periodo entre los 11 a los 15 días del crecimiento embrionario. La relación de este caso con otros publicados es objeto de discusión.

Translation by José F. Nonidez  
Cornell Medical College, New York

## CASE OF UNILATERAL ABSENCE OF KIDNEY, URETER AND DISTAL PART OF UTERINE TUBE IN THE RABBIT

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ONE FIGURE

### DESCRIPTION

The specimen under consideration is a female with the right side of the urinogenital system normal, save that the right kidney is larger than is usually the case in an animal of the same size. The description of the urinogenital system is as follows:

Left kidney absent; no trace of left ureter; that there was no opening into the bladder on the left side was determined by injecting a semifluid colored mass into the bladder. The right ureter was partially filled.

Left suprarenal body present in normal position, but rotated on its axis through  $90^{\circ}$ ; supplied with blood from the supracrenolumbar artery.

Left vesicorectal fold present, but contains only the left umbilical artery and does not connect with the vagina.

Right kidney measured 3.4 cm. by 2.2. cm. by 0.7 cm., slightly larger than in normal rabbits of the same occiput-to-tail measurement.

Right ureter normal in position; opening into right side of base of bladder along vesico-uterine fold.

Right suprarenal body normal.

Left ovary slightly longer than right; normal in position. Fimbria, supported by mesovarium, overhangs ovary anteriorly. Oviduct leads to a point near the posterior pole of ovary and ends blindly in a small knob. A narrow mesenterial fold binds oviduct to ovary.

Left ovarian ligament passes from posterior pole of ovary to the point usually reached by left round ligament.

Vagina lies completely to the right. No trace of left uterus.

Left ovarian artery normal in relation. Left ovarian vein passes caudad to join the inferior vena cava just past the point where median sacral artery arises from aorta.

Right portion of genital system normal.

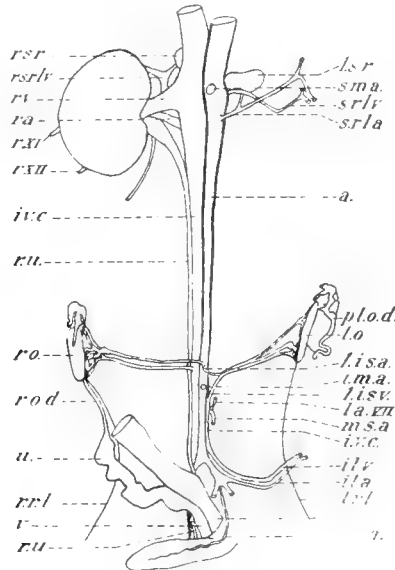


Figure 1.  $\times \frac{1}{2}$  natural size

#### ABBREVIATIONS

<i>a.</i> , aorta	<i>r.a.</i> , renal artery
<i>i.l.a.</i> , iliolumbar artery	<i>r.o.</i> , right ovary
<i>i.l.v.</i> , iliolumbar vein	<i>r.o.d.</i> , right oviduct
<i>i.m.a.</i> , inferior mesenteric artery	<i>r.r.l.</i> , right round ligament
<i>i.v.c.</i> , inferior vena cava	<i>r.s.r.</i> , right suprarenal body
<i>l.a.VII</i> , seventh lumbar artery	<i>r.s.r.l.v.</i> , right suprarenolumbar vein
<i>l.i.s.a.</i> , left ovarian artery	<i>r.u.</i> , right ureter
<i>l.i.s.v.</i> , left ovarian vein	<i>r.v.</i> , renal vein
<i>l.o.</i> , left ovary	<i>r.XI</i> , eleventh rib
<i>l.r.l.</i> , left round ligament	<i>r.XII</i> , twelfth rib
<i>l.s.r.</i> , left suprarenal body	<i>s.m.a.</i> , superior mesenteric artery
<i>l.u.a.</i> , left umbilical artery	<i>s.r.l.a.</i> , suprarenolumbar artery
<i>m.s.a.</i> , median sacral artery	<i>s.r.l.v.</i> , suprarenolumbar vein
<i>p.l.o.d.</i> , proximal part of left oviduct	<i>u.</i> , right uterus
<i>r.</i> , rectum	<i>v.</i> , vagina

## PROBABLE ORIGIN OF THE ANOMALY

In studying the condition it occurred to the writer that it might be possible to determine approximately the age of the embryo when the defect arose. The association of the presence of a perfect ovary and a normal proximal part of the uterine tube with absence of the distal part of the same tube and total absence of the kidney and ureter on the same side would give such a clue. The anterior growth of the ureter and the posterior growth of the müllerian duct have both suffered interference.

According to Minot ('05), the ureter first appears at eleven and one-half days in a 6-mm. embryo rabbit, and at fifteen days in a 12- to 13-mm. embryo the müllerian ducts are present, lying on the coelomic side of the wolffian ducts, extending only a short distance and opening into the coelom at the cephalic part of the wolffian body. In man, according to Felix in Keibel and Mall ('12) "At the very time when the posterior end of the groove is separating from the epithelium it begins to grow out caudally and in this process we come to the development of the distal portion of the müllerian duct. It is formed by the gradual outgrowth of the tip of the cornet." Some interference with growth starting near the caudal end of the mesonephric duct before eleven and one-half days and reaching near the anterior end of the mesonephros at about fifteen days must have been responsible for the condition. If this process is in the nature of an extension of the natural degeneration of the mesonephros the latter must have been markedly premature as well as extensive. This is shown by the normal time of onset of the degeneration. Thus Felix and Bühler, in Hertwig's Handbook ('04), put the degeneration in an 18- to 20-mm. embryo at eighteen days. Bremer ('16) states that the placenta takes over the function of the mesonephros at an age corresponding to 21 mm., when the mesonephros begins to degenerate and ultimately loses all active glomeruli at 40 mm. The tubular degeneration is still later. Thus Felix ('04) says, "Beim Kaninchen beginnt die Rückbildung des primären Harnleiters bei 5 cm. langen Embryonen und zwar in der Mitte und schreitet von da rasch in kranialer und kaudaler Richtung vor und führt zum vollständigen

Schwund des primären Harnleiters (Langenbacher, '82).'' The method of degeneration is, however, similar to that which I have visualized in this case.

#### COMPARISON

Now, if we compare a few cases of a somewhat similar nature, Hunt ('18) describes the absence of the right kidney in a male domestic cat. The right ureter was present, 1.5 cm. long. The genital system and suprarenals were normal. Since the ureter appears in this specimen, the process has not occurred early enough to cause the inhibition of the evagination. The mesonephric duct is also retained normally as the genital duct. Therefore, the destructive agent acted on the nephrogenic mass shortly after the ureter began its growth and did not involve the mesonephric ducts, but only the part of the nephric mass that normally forms the mesonephric and metanephric glomeruli. The next case is described by Lyon ('17) in man. The right kidney, ovary, and uterine tube were absent. The right suprarenal was present, as was also the right ureter, which was connected with the bladder, although no opening from it into the bladder could be found. The interior of the right ureter contained a small amount of granular debris. The uterus was small with only the left tube. This case is one in which another consideration might appear, namely, the division of the mesonephros into a cranial and a caudal part, as shown in Minot's ('10) figures for the pig. The genital gland and the whole duct in Hunt's case and part of the duct in my case were present. In Lyon's case the ovary and the whole tube were missing. These parts are closely associated with the cranial part of the mesonephros. Therefore, my case shows more interference with growth in the region of the caudal mesonephros; Lyon's in the cranial part; while in Hunt's the involvement was caudal, but relatively less. Brown's ('94) case in man shows absence of the right kidney, but the right ureter is present. Harrison ('94) describes in the rabbit an absence of the left kidney and ureter. Both ovaries are present, but uterine tubes and uteri are absent. Here both sides are involved anteriorly. Reterer and Rogers

('93) describe a case in the rabbit with an anomalous condition on the right side similar to the condition described in this paper on the left side (reported by Radasch, '08).

Radasch ('08) gives an extensive review of the literature up to 1908 and concludes that the process causing the loss of kidney is due either to the failure of the ureteric evagination or its retrogression after its appearance. In the case he describes in the cat the presence or absence of the ovary and part of the tube on the right side was not capable of determination, but the right ureter was absent. This would place the condition in a similar class to the one described in this paper.

#### SUMMARY

A case is described in a rabbit of the absence of the left kidney and ureter, part of the left uterine tube, and the left uterus. By taking into consideration the conditions during embryonic development, the origin of the anomaly is placed at probably from eleven and one-half to fifteen days' embryonic growth. The relationship of this to other reported cases is discussed.

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Resumen por el autor, Warren Lewis.

### La cualidad adhesiva de las células.

Es evidente que las células que emigran sobre la superficie inferior del cubreobjetos en los cultivos de tejidos son adhesivas; de otro modo, cuando se emplea un medio de cultivo líquido, caerían al fondo de la gota, puesto que son más pesadas que el medio. Las células son adhesivas a consecuencia del mismo material de que están formadas, del mismo modo que la cola es pegajosa. Sin esta cualidad adhesiva al vidrio y algunos otros sólidos, no podrían emigrar. No existe razón alguna para creer que desarrollan esta cualidad cuando abandonan el trozo cultivado y vienen a ponerse en contacto con el vidrio. Esta cualidad adhesiva varía un tanto, puesto que las células pueden redondearse y desprenderse del cubreobjetos. Este fenómeno es pasivo y no corresponde de modo alguno a la sensibilidad que exhiben ciertos animales para los sólidos, conocida con el nombre de estereotropismo. Las células se adhieren no solo al vidrio y otros sólidos, sino también entre sí en grado variable. Esta cualidad adhesiva juega un papel muy importante en la persistencia del contacto de las células y los diversos tejidos, no solo durante la segmentación y en el embrión antes de formarse las fibras intercelulares, sino también en el adulto. La existencia de continuidad protoplásmica actual, es muy dudosa en la mayor parte de los tejidos, tales como el mesenquima, epitelio y músculo. De tal modo que si las células de nuestro cuerpo perdiesen súbitamente su cualidad adhesiva, nos desintegraríamos repentinamente y los varios tipos celulares formarían una corriente mezclada de ectodermo, músculo, mesenquima, hígado y otros varios tipos celulares.



## THE ADHESIVE QUALITY OF CELLS

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From observations on the behavior of cells in tissue cultures, it seems to me that the causative factors in the phenomena exhibited by the cells in their migration out from the explant along solid supports resolve themselves into two separate categories: 1) a natural stickiness or adhesiveness which these cells appear to have for certain solids and for each other; 2) the forces that cause them to migrate. Concerning the first very little has been written, although I think we have all been aware that many cells are sticky. We have long known, for example, of the adhesive quality of white blood-cells for glass. The importance of this adhesive quality in the various body cells, however, has been almost entirely ignored. Yet most of our tissues and organs are made up of cells that are merely stuck together. Were these various types of cells to lose their stickiness for one another and for the supporting extracellular white fibers, reticuli, etc., our bodies would at once disintegrate and flow off onto the ground in a mixed stream of ectodermal, muscle, mesenchyme, endothelial, liver, pancreatic, and many other types of cells.

It will seem to many that I am overlooking a very important consideration, namely, the so-called syncytial relations supposed to exist in many tissues, such as the smooth muscle, heart muscle, and especially the mesenchyme, as also the ectoderm, by virtue of its so-called intercellular bridges. I am convinced, after the study of the behavior of these cells in tissue cultures of chick embryos, that the outgrowths of these tissues do not form syncytia. From this it would appear highly probable that they do not form syncytia either in embryos of four to ten days' incubation (the usual ages from which the tissues were taken for cultures) or in the adult, and that this holds true not only for birds,

but for mammals as well. Some of the evidence for the non-synectial nature of mesenchyme has already been published<sup>1</sup> and that for heart muscle, smooth muscle, and ectoderm is nearly ready for publication.

It is almost self-evident that the cells that migrate out on the under surface of the cover-glass, in cultures where a fluid medium is employed, are sticky for glass. They are often apparently quite firmly adherent, and such preparations can be washed and manipulated in various ways and with various fluids, or even fixed and killed by different agents, without disturbing their attachment. They can even be poked with a needle or centrifugalized, and yet the cells will not be dislodged. Of course, cells do frequently become detached from the glass through manipulations of various sorts, or they may at times round up and drop to the bottom of the hanging-drop, but even after rounding up many still remain attached. Not only the bodies, but the cell processes as well possess this adhesive quality, and it not infrequently happens that an outgrowth, contracting back towards the explant, snaps apart some of the more extended processes at the periphery, leaving slender, isolated fragments of cytoplasm adhering to the cover-glass. The adhesiveness of such fine processes, some of them so thin as to be almost invisible, can scarcely be explained on any other ground than stickiness. It is highly improbable that the adhesion is maintained by some sort of suction apparatus. I do not quite see how it could be proved that the cells are or are not sticky for glass before they come into contact with it, and there is no particular reason for believing that they develop such a special adhesive quality as they leave the explant and come in contact with the cover-slip.

Cells are not only sticky for the cover-glass, but for each other as well. In the body of the embryo and in the tissue explant such cells as endothelium, mesothelium, and the endodermal lining of the intestine are undoubtedly merely adherent to their neighbors, but they retain their adhesive quality in the outgrowths and, in addition, stick to the cover-glass. I believe the same thing is true for those cells that have hitherto been supposed to form synectia.

<sup>1</sup> Lewis, W. H. Is mesenchyme a synectium? *Anat. Rec.*, vol. 23, 1922.

We may in time be able to measure the force of the adhesions in some way, as by utilizing the centrifugal force necessary to dislodge them, but so far even the high speed of the ordinary centrifuge fails to move those that are flattened out to any extent from their attachment to the cover-glass. The fact that cells do move and shift about, spread out very flat, or round up and drop off indicates that the factors of cohesion and surface tension of the protoplasm are constantly at work in altering the extent and position of the adherent areas, both on the cover-glass and on the other cells. Such changes may very well take place without altering the natural adhesiveness of the surface of the cells, but it is entirely possible that the degree of stickiness also may vary. One wonders whether this sticky quality is dependent upon the composition of the protoplasmic surface membrane that limits all cells or upon some sort of substance that is secreted by the cell as one of the products of its metabolism. The so-called cement, so commonly described as existing between various types of cells, may possibly be an adhesive substance. This is usually recognized by its power to reduce silver nitrate. Now, most living cells in our cultures show a slight browning over their entire surface with silver nitrate, while dead cells do not; it is therefore possible that the silver nitrate is reduced by the living protoplasm and not by any substance on its surface. The heavy lines seen at the contact edges of cells seem to be, in part at least, an optical effect of looking edgewise at a thin granular film.

The important rôle which this adhesive quality plays in the early stages of development, before the formation of extracellular fibrils of any sort, can scarcely be overestimated. Without it multicellular organisms could not exist. When it is repressed, as was done by Herbst ('00)<sup>2</sup> in the segmentation stages of certain marine animals, by eliminating calcium salts from the sea-water, the blastomeres fall apart and round up and further development of the embryo becomes impossible. In the blastula and gastrula stages the only other factor that might play a rôle in keeping

<sup>2</sup> Herbst, Curt. Ueber das Auseinandergehen von Furchungs- und Gewebezellen in kalkfreiem Medium. Arch. f. Entw. Mech., Bd. 9, 1900, S. 424.

the cells in position is the pressure from without of an external membrane inclosing the whole organism. I am eliminating entirely the conception of intercellular bridges, which has been postulated by Hensen<sup>3</sup> and others, and have adopted the opposite extreme, which may seem to many as far from the truth as the idea of universal cell continuity. In the early embryonic stages, before the formation of extracellular fibrils and other supporting and binding substances, whether these be in the form of gels or of fibers, all the tissues are held together by adhesion or a combination of adhesion and interlacing of elongated and branched cells. In later stages and in the adult the extracellular supporting substances play an important rôle in holding cells and tissues together, but the intercellular adhesions are probably still more important.

The most obvious deduction to draw from the known facts is that the cells in tissue cultures are sticky as regards certain solids, and that this is a natural characteristic. They are sticky because of the very material of which they are composed, just as egg albumen, glue, and mucilage are sticky. If, then, we admit that the cell surface is naturally sticky for glass, we can scarcely consider that adhesion of the cells to solids is a form of tropism. 'Tropism' as the term is usually employed, applies to the phenomenon observed in living organisms of moving toward (positive) or away from (negative) a focus of light, heat, a solid body (stereotropism), or other stimulus. The term 'stereotropism' has frequently been employed to indicate a reaction exhibited by all of the cells in tissue cultures that migrate out from the explants on solid supports. Harrison ('11, '14)<sup>4</sup> and others have observed that cells that migrate out from the explant always utilize some solid support, such as the cover-glass, fibrin threads, spider-web, fibers of various sorts, or even the surface film of the fluid hanging-drop. None of the ordinary tissue-cells are able to swim about in fluid media. The idea

<sup>3</sup> Hensen, V. Ueber die Entwicklung des Gewebes und der Nerven im Schwanz des Froschlarve. Virchow's Arch., Bd. 31, 1864.

<sup>4</sup> Harrison, R. G. The reaction of embryonic cells to solid structures. Jour. Exp. Zool., vol. 17, 1914. Science, vol. 34, 1911.

underlying the use of the term stereotropism, as applied to the cells in tissue cultures, seems to be that there is some sort of positive reaction on the part of the cells toward the solid. Cells migrate only on solids; therefore it is assumed they are in some way attracted by solids. The following quotation from Harrison ('14, p. 541) indicates the present attitude on the subject:

"While it must therefore be admitted that chemical stimuli may play an important part in influencing the movements of cells in simple cultures, as Burrows has pointed out, the facts show that the cells are also stimulated by solids as such and respond to them by an orienting movement. Response to tactile stimuli is of such general occurrence in animals that there is nothing anomalous in the manifestation of the same kind of sensitiveness in cells."

We say that cells are stereotropic because they have only been observed to migrate on solids, and that they migrate on solids because they are stereotropic. To say that cells are stereotropic does not offer any explanation for the fact that they migrate only on solids. On the other hand, the fact that they can only migrate on solids does not necessarily mean that they are stimulated by solids as such, nor is there any reason to believe in a sensitiveness of cells that corresponds in any way to the response of animals to tactile stimuli. The adhesion of cells to solids is a passive phenomenon. Since cells are heavier than the fluid medium, they could not migrate on the under surface of the cover-slip unless they were adherent.

Concerning the factors which cause the cells to migrate out from the explant along the cover-glass or other solids, on the surface film of a liquid drop, or on other cells (which behave as solids), there is still much uncertainty. The work of Tait ('18)<sup>5</sup> and Leo Loeb ('20)<sup>6</sup> indicates that the progressive movements

<sup>5</sup> Tait, J. The capillary phenomena observed in blood cells, etc. *Quart. J. Exp. Physiol.*, vol. 12, 1918.

<sup>6</sup> Loeb, Leo. The movements of the amoebocytes and the experimental production of amoebocyte (cell-fibrin) tissue. *Wash. Univ. Studies*, vol. 8, Scientific Series no. 1, 1920.

of the cells may depend upon local variations in the metabolism which produce local changes in the fluidity and surface tension of the cells, while the observations of Burrows<sup>7</sup> suggest that the interchange of substance between explant and medium may offer a basis for a differential localization of metabolism.

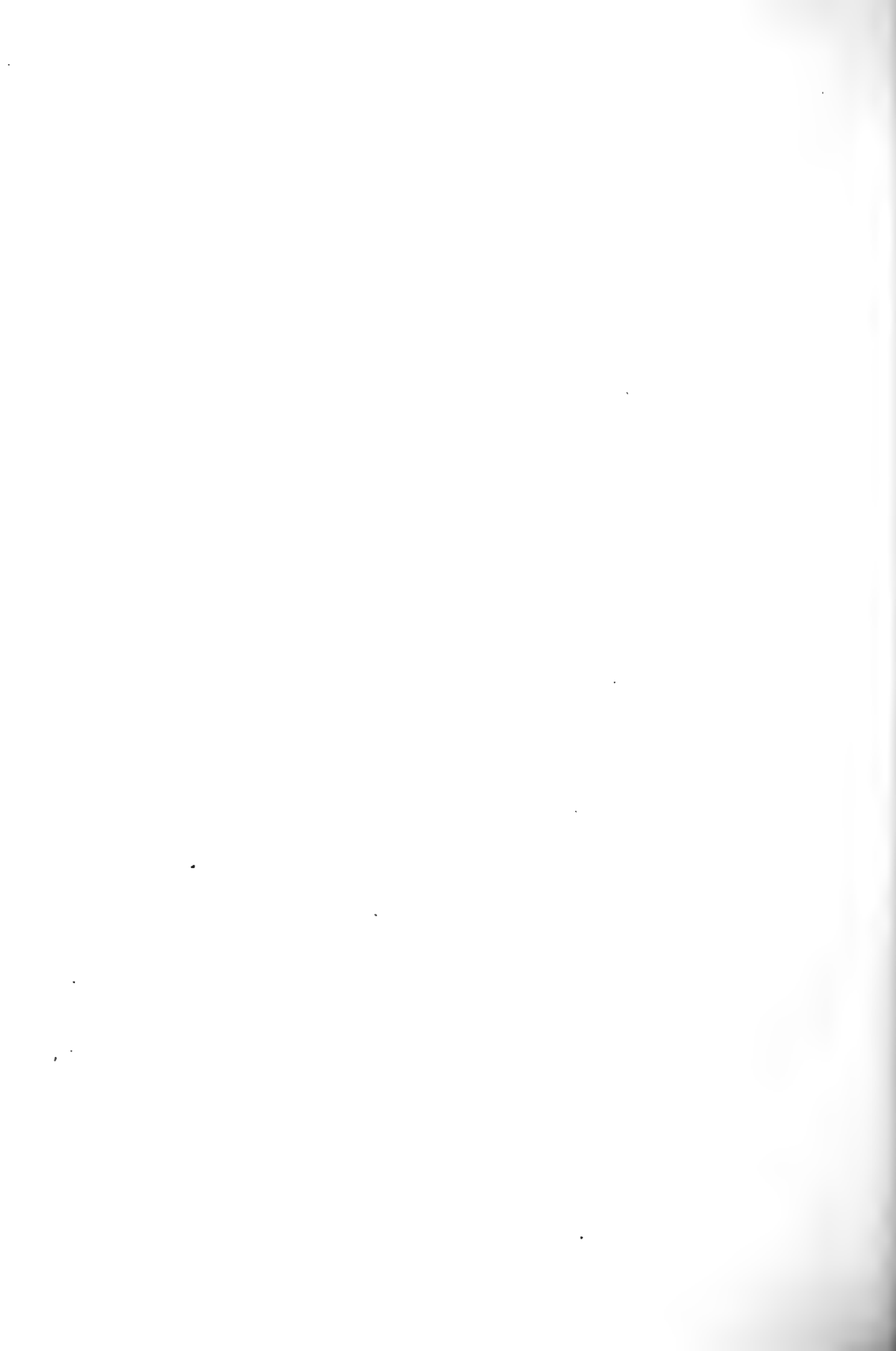
<sup>7</sup> Burrows, M. T. The tissue culture as physiological method. Trans. Cong. Amer. Physicians and Surgeons, vol. 9, 1913.

# THE ANATOMICAL RECORD

EDITOR  
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VOLUME 24  
JULY, 1922—JANUARY, 1923

PHILADELPHIA  
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY





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## PROMPT PUBLICATION

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Resumen por el autor, Robert Chambers.

Un nuevo aparato y métodos para la disección e inyección de las células vivas.

El autor presenta una descripción detallada de un micro-manipulador mecánico basado en un nuevo principio, de simple construcción y que posee rasgos que permiten al operador mover microagujas y pipetas para la disección e inyección de las células vivas y para aislar bacterias con facilidad y precisión. El principio del aparato es un sistema de barras rígidas reunidas mediante muelles sobre los cuales actúan tornillos para separarlas. Una forma del instrumento puede sujetarse mediante pinzas directamente sobre la platina del microscopio, la otra puede fijarse sobre un pilar rígido atornillado en una base metálica sobre la cual se fija el microscopio mediante pinzas.

El autor incluye una descripción de los métodos para obtener microagujas y pipetas de vidrio, así como la de la cámara húmeda en la cual se llevan a cabo las operaciones de disección e inyección. Discute también el empleo de condensadores de larga distancia focal. Así mismo, describe un método para obtener de un modo simple y efectivo un aparato para la microinyección, el cual se emplea con el micromanipulador. El trabajo lleva un apéndice en el que presenta una revisión histórica de los aparatos empleados en la microdisección hasta el presente. La ventaja del micro-manipulador descrito en este trabajo sobre los fabricados hasta el presente es su simplicidad, su firmeza, su exactitud de movimiento y la falta total de movimientos perdidos aunque se use durante mucho tiempo.

Translation by José F. Nonidez  
Cornell Medical College, New York

## NEW APPARATUS AND METHODS FOR THE DISSECTION AND INJECTION OF LIVING CELLS

ROBERT CHAMBERS

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FIVE FIGURES

### INTRODUCTION

Operative work on the living cell has long been the aim of investigators in cytology and in experimental embryology. It was, however, not till Barber developed his method that any serious attempt could be made to dissect cells under magnifications high enough to enable one to observe in detail the various steps of the operation. The big feature of his method, aside from the making of needles and pipettes stiff and yet fine enough to puncture red blood corpuscles, consists in his moist chamber, which allows the needle tips to be operated in a drop hanging from a cover-slip in the chamber. This method eliminates all obstacles between the objective and the cover-slip, thereby permitting the use of the highest-powered objectives. Unfortunately, his instrument for manipulating the needles, unless very skillfully made, has too much lost motion, and wear and tear soon render the movements jerky and undependable.

Barber uses his apparatus principally for the isolation of bacteria. In 1912 Kite (Kite and Chambers, '12) applied Barber's method to cytological investigation. The difficulty of handling Barber's apparatus limited the number of investigators in this field and as the work in microdissection progressed the need of a more accurate and simple instrument became imperative.

The instrument described in this paper, a preliminary account of which has been published ('21), has the following advantages over any instrument hitherto made: a) simplicity of construction,

b) no lost motion through wear and tear, c) accurate and continuous control of the movements of the needle or pipette tip in any direction under the highest magnifications of the microscope, d) maintenance of the needle tip in one plane while it is being moved back and forth in any of the three directions, and e) existence of preliminary adjusting devices which facilitate placing the needle or pipette quickly into position.

The basic principle of the instrument consists in rigid bars which are screwed apart against springs. The movements imparted are in arcs of a circle having a radius of about two and a half inches. As the extreme range of movement of the fine adjustments is only 2 mm. (of which only one is necessary) the curvature of the arc is unnoticeable.

The movements performed by the instrument are so accurately controlled that one can readily carry out such delicate operations as puncturing mammalian blood corpuscles, tearing off the sarcolemma of a muscle fiber, drawing out nuclear chromatin strands and even cutting up the chromosomes of insect germ cells. The glass needles used for these operations taper rapidly to a point invisible under the oil immersion objective. With the micropipette, the bore of which need be no larger than one micron in diameter, one can either inject substances into or withdraw material from a cell.

For the isolation of bacteria the instrument is not only steadier than Barber's apparatus but has new features which facilitate greatly the method of procedure. Its application to bacteriological purposes is more specifically dealt with in the *Journal of Infectious Diseases*.

I take this opportunity of expressing my deep obligation to Mr. W. H. Farnham, mechanic in the department of Chemical Engineering in Columbia University, to whose skill and faithful workmanship the practical evolution of the instrument is due. I wish especially to acknowledge assistance from Dr. Milton J. Greenman of The Wistar Institute and Dr. C. V. Taylor of the University of California. I wish also to express my appreciation to many friends for valuable suggestions. The principle involved in the construction of the micromanipulation instrument is patented.



angled isosceles triangle the base of which is a straight line joining the centers, *E* and *F*, of the two springs holding the three bars, *A*, *B*, and *C*, together. The shank of screw, *G*, passes through a large hole in bar, *C*, and is screw-threaded in bar, *B*. Turning it spreads apart bars, *A* and *B*, and imparts an arc movement to the needle tip at *D* at right angles to that procured by turning screw, *H*.

The movement in the vertical plane at right angles to the aforementioned movements is produced by screw, *I* (fig. 1, a), which is screw-threaded in a rigid vertical bar, *J*, and abuts against a vertical extension, *K*, of bar, *C*. The extension, *K*, is parallel to the bar, *J*, and is connected to it at its top by means of a solid spring hinge. Turning screw, *I*, spreads apart bars, *J* and *K*, and lifts the whole combination (*A*, *B*, and *C*) and imparts an arc movement in the vertical plane to the tip of the needle at *D*. To procure a vertical movement, the tip of the needle at *D* must lie in the same horizontal plane, *L-D*, with the spring fastening *K* and *J* together. When screw, *I*, is turned, the needle tip will then move in an arc, *Y* to *Z*, more nearly vertical than any other arc on the same circumference of which the point, *D*, is the center.

There are two models of the micromanipulator. One is fitted with a clamping device with which it can be fastened directly to the front of the microscope stage (fig. 2; cf. fig. 3, e).<sup>1</sup> The other is fastened to a rigid pillar rising from a large metal base on which the microscope is clamped (fig. 3, a). The horizontal bars of the instrument extend diagonally across the corner below the level of the stage. They do not interfere with the substage accessories of the microscope nor with any of the known types of mechanical stages.

The necessity of having one or two instruments is, of course, conditioned by the type of work to be done. For picking up bacteria one is sufficient. For microdissection in experimental embryology a great deal can be done with one instrument, but for cell injection in general and for tissue cell dissection two

<sup>1</sup> Steadiness may be assured by a brace, one end being screwed to the rigid vertical part of the instrument and the other end to the foot of the microscope.



instruments are indispensable so that two needles or a needle and a pipette may be manipulated simultaneously. When two instruments are to be used both must be placed at the front of the microscope so that the needles may extend, side by side, into the moist chamber from the front. As the horizontal bars of each instrument extend diagonally under the microscope stage

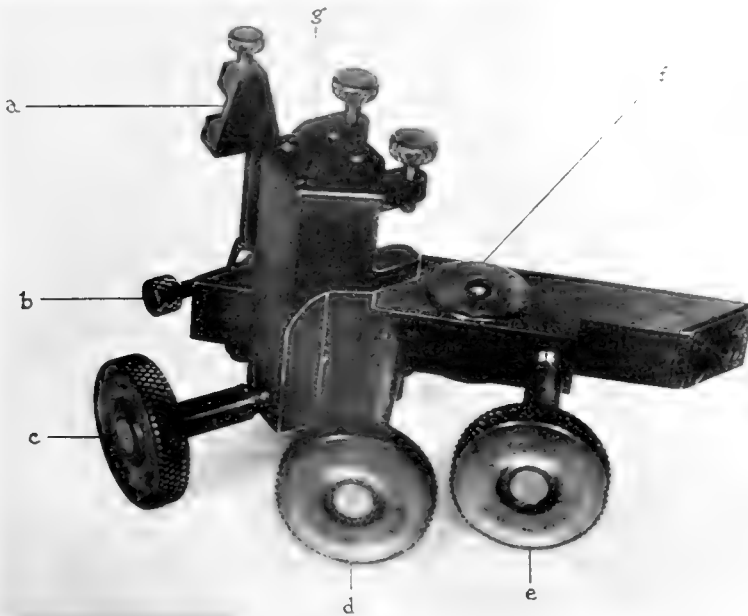


Fig. 2 Left-handed micromanipulator to be clamped to microscope stage. *a*, needle carrier with clamping screw; *b*, screw to clamp post of needle carrier; *c*, screw for up-and-down movement; *d* and *e*, screws for lateral movements; *f*, disc guide for the horizontal bars; *g*, stationary or rigid part of instrument with lugs by means of which instrument is clamped to microscope stage. Screw, *b*, clamps the coarse adjustments.

one must be a mirror image of the other. According to their position with respect to the microscope these two models have been designated as left-handed and right-handed. For bacteriological work, where it is more convenient to work from the left, the right-handed model is to be preferred as it can be swung around and fastened to the left side so that the pipette may

extend into the moist chamber from the left. For cytological work, if one desires to have only one instrument, it is advisable to secure the left-handed form and to use it as shown in figure 3, e. The mechanical stage may then be operated with the right hand and the instrument with the left. Eventually this instrument may be supplemented with a right-handed form to be clamped to the stage or attached to a pillar. When a pair of instruments is to be used the best combination is a left-handed one clamped to the microscope and a right-handed one attached to a pillar (fig. 3). This allows one to hold the tissue on which one is operating with one instrument while the microscope is being temporarily removed for renewing the pipette of the other (see page 14).

#### THE SETTING UP AND THE WORKING OF THE INSTRUMENT

Figure 3 shows two instruments in place ready for work. They should be as close together as possible so that the open end of the moist chamber need not be too wide to accommodate the needles. This leaves ample room on either side for the attachment of a mechanical stage on the microscope.<sup>2</sup>

The instrument is provided with means for a preliminary adjustment of the needle in any direction. By these means the needle tip can be quickly centered in the field of a low-powered objective and raised close to the hanging drop in which it is to operate. Before centering the tip the bars which control the fine adjustments must be put into a state of tension by giving a few turns to the milled heads of each of the three screws. The instrument is now ready for action.

The milled heads of the screws which control the lateral movements are provided with holes for rods to be used as levers. A most useful accessory is a wire-wound flexible shaft about 2 feet 6 inches long (fig. 3, c) with a milled head at one end (fig. 3, d) and the other end attached to the screw controlling the up-and-down movement. Curving the shaft around one side of the microscope brings the control of this screw, which is the one most

<sup>2</sup> In the case of the Bausch & Lomb and Spencer stages, it may be necessary to replace the screw clamping the front end of the stage by one with a smaller head.

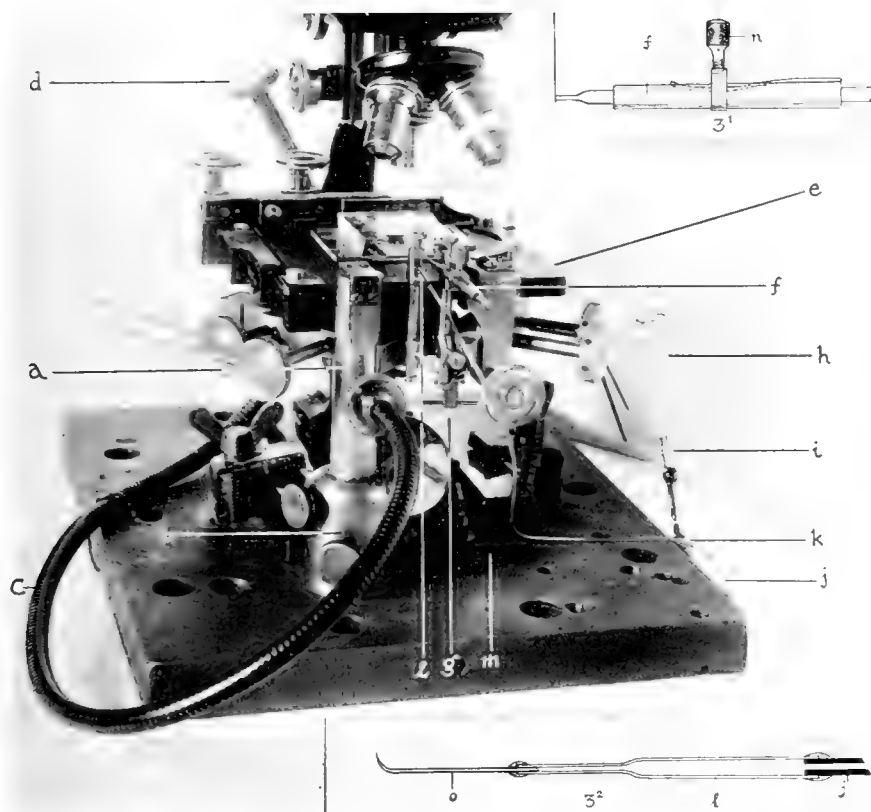


Fig. 3 Microscope with two micromanipulators and the microinjection apparatus in place. *a*, right-handed manipulator on pillar set in collar, *b*, fastened to base on which microscope is clamped; *c*, flexible shaft attached to screw for up-and-down movement with its milled head at *d*. (Note that screws for lateral movements are controlled by levers.) *e*, left-handed manipulator clamped to left front of microscope stage. In its needle carrier is clamped brass collar, *f*, within which shaft of needle slides. (See detail in fig. 3¹.) The coarse adjustment for raising and lowering needle carrier is done by screw, *g*; that for the lateral movements is done by turning the post on its axis.

Injection apparatus. *h*, Luer syringe set in its butt, *i*, cemented to curved brass tube, *j*. This is clamped to base at *k*. Its other end is cemented into glass tube, *l* (see detail in fig. 3²), clamped in needle carrier of the right-handed manipulator, *a*.

Most of the holes in the base are unnecessary. Foot of microscope is held by two screw clamps. The adjustable guide, *m*, keeps microscope in proper alignment.

Fig. 3¹ Detail of brass collar (*f* in fig. 3) which facilitates in-and-out movement of needle or pipette; *n*, screw which presses on a spring to clamp the needle in the collar.

Fig. 3² Detail of glass tube of injection apparatus (*l* in fig. 3) cemented on brass tube, *j*; *o*, shank of micropipette cemented into end of glass tube. The pipette is readily changed by softening the sealing wax which holds it.

frequently used, close to that of the fine adjustment of the microscope. The shaft also facilitates the use of both hands for the various movements of the one instrument.

Another useful accessory is a brass collar  $1\frac{1}{2}$  inch long (fig. 3) with a spring which projects into its lumen through a slot. The shaft of the needle is slipped through the collar and the screw, clamping the spring, tightened sufficiently to enable one to slide the shaft evenly. The collar is then clamped into the needle carrier of the instrument. This arrangement facilitates sliding the needle into or out of the moist chamber without danger to the tip of the needle.

The micromanipulator is intended to be used with the mechanical stage of the microscope. The mechanical stage moves the moist chamber (fig. 3). As the cell or tissue to be dissected lies in a drop hanging from the roof of the chamber, the motion imparted by the mechanical stage moves the cells against the micro-needle. Indeed, most of the dissection, where a single needle is used, is done by first bringing the needle tip into the cell and then dragging the cell away by means of the mechanical stage.

The horizontal movements of the micromanipulator are used mostly for the purpose of bringing the tip of the needle accurately into a desired spot in the field of the microscope preparatory to the actual operative work. In order to insure the greatest possible steadiness to the vertical movement, the part of the instrument which imparts this movement adjoins and is manipulated from the stationary and rigid part of the instrument. To make this possible the present design incorporates a theoretical error which can be understood from figure 1, a. Turning screw, *I*, to produce the vertical movement throws the combination of bars *A*, *B*, and *C*, out of the horizontal, and it is these bars upon which the lateral movements of the needle depend. However, the angle at which these bars are placed minimizes the error so that it is unnoticeable.

Guides exist in the instrument to insure a true travel of the bars as they spread apart or come together. The guide for the bar which produces the vertical movement consists of a depression in the stationary part of the instrument into which the verti-

cal bar fits. The guides of the lateral movements are two metal discs which can be tightened or loosened by screws. The upper one is seen in figure 2, f. They correct two possible errors which may occur on reversing the direction of movement, viz., a dropping of the needle or pipette out of focus and a shifting to one side.

The first error can be corrected by tightening one or both of the guides; the second, by loosening them. The guides, therefore, must be neither too tight nor too loose. The first error is the more serious of the two. It is due to an unequal tension in the springs which throws the tip of the moving screw to a different spot on the bar against which it abuts. If this be not corrected, the screw will in time wear a depression in the brass bar that is out of center thus accentuating the error. The second error is due to the guides being too tight so that they bind and prevent the bars from making a true return. If not corrected, this error will be gradually eliminated with the wear of the frictional surfaces.

By an accidental knock the horizontal bars of the instrument may be jarred out of place or the fine adjustment screws injured. If the upper and lower surfaces of the horizontal bars are not flush loosen the guide discs (fig. 2, f) also the screws of the springs on the ends of the bars and, with a wooden mallet, gently hammer the bars till they are flush. Then tighten the guide discs to keep the bars flush and carefully tighten the screws of the spring. If the screws have been bent by the accident they must be changed otherwise tightening them will again pull the bars out of place. If the guide discs are bent they also must be changed. A more serious accident is when the fine adjustment screws are injured. The steel shafts of the screws may be bent or they may have cut into the brass so as to loosen the threads. This tends to throw the shaft of the screw out of center. In such a case somewhat larger screws must be made and accurately centered opposite the bar against which it abuts.

#### THE SUBSTAGE CONDENSER AND THE METHOD OF MAKING BARBER'S MOIST CHAMBER AND GLASS NEEDLES

For critical illumination the height of the moist chamber must be equal to the working focal distance of the substage condenser.

The Abbe condenser can be used by removing the top lens. The focal distance of the remaining lens is almost one inch. In the Bausch and Lomb microscope the substage can easily be arranged to raise this lens sufficiently to have at least half its focal distance above the surface of the stage. This is ample, for one seldom requires a moist chamber as high as half an inch. The focal distance of this lens can be reduced and its illuminating power correspondingly increased by placing the lens of a 10X dissecting lens on top of it. This combination has a focal distance of about  $\frac{3}{8}$  of an inch and, if the substage can be raised to bring the top lens flush with the upper surface of the stage, all of this distance may be used for the height of the moist chamber. Better results are secured with a triple lens condenser with its top lens removed. Such a condenser from Leitz which I am using has a working focal distance of  $\frac{3}{8}$  of an inch. One may also use condensers which are made with a specially long working distance for projection apparatus, in which a cooling trough is placed between the condenser and the slide.

If the working focal distance of the condenser be less than  $\frac{3}{8}$  of an inch, it is well to have two moist chambers, one for critical work and the other, from  $\frac{3}{8}$  to  $\frac{1}{2}$  an inch high, for ordinary work. This is advisable, because it is easier to make needles for the higher chamber.

The moist chamber is of glass (fig. 4). The base is a thin glass slide about  $2\frac{3}{8}$  x 2 inches in size. The sides consist of strips of plate glass about  $1\frac{7}{8}$  inches long and  $\frac{1}{4}$  inch wide, and of a height determined upon by the available condenser. One end of the chamber is closed with a strip of glass of the same height as the sides and backed by another strip a fraction higher, in order to prevent a cover-slip from sliding beyond it. The trough of the chamber should be from  $\frac{3}{4}$  to  $\frac{7}{8}$  of an inch wide. The strips are cemented with any ordinary glass cement. Heated Canada balsam serves well. Near the closed end of the trough a small strip of glass should be cemented across the trough to provide a well for water. When cementing the long strips to the base, care must be taken to have the top surface of the strips horizontal. This may be done while the cement is still soft by

focusing on the upper surface of the strips and by manipulating the strips until all parts of their surfaces lie in one focal plane.

The well in the chamber is to be filled with water and, in order to distribute the moisture throughout the chamber, strips of blotting-paper should be placed along the sides of the trough with the inner end in the water well. One may substitute for the well strips of blotting-paper laid across the trough. This moist chamber is designed for cover-slips of a size 24 x 40 mm. The cover-slip is sealed on the chamber with vaseline. Square

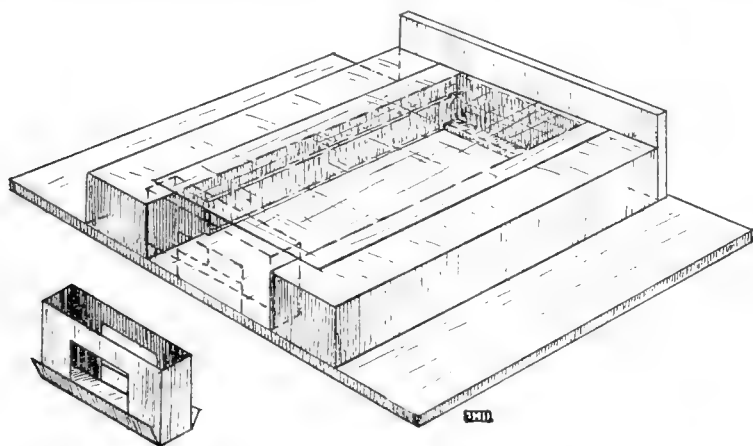


Fig. 4 Moist chamber and cardboard trough for closing open end of chamber. When the needles are in place (cf. fig. 3), the trough is placed over shanks of needles (dotted lines at open end of chamber) and filled with vaseline.

cover-slips may also be used, if the rest of the chamber be roofed with other strips of cover-glass.

The moist chamber is open at one end to permit the entrance of the microneedles or pipettes. To prevent undue evaporation, especially when a preparation is to be left over night, the open end may be temporarily closed by means of a paraffined thin cardboard trough of a shape shown in figure 4. The trough is placed over the shafts of the needles and filled with soft vaseline containing a few threads of cotton to give substance to the vaseline. The vaseline closes around the shafts of the needle and seals the opening of the chamber without interfering with the movement

of the needles. To prevent the vaseline from spreading on the floor of the moist chamber, it is well to have a shallow pan of cardboard set under the shafts of the needles for the trough to rest upon.

The hanging-drop containing the cells or tissue to be operated upon is placed on the cover-slip which is then inverted over the moist chamber.<sup>3</sup> To prevent the vaseline from spreading on the cover-glass and from contaminating the hanging-drop, a thin film of melted paraffin may be spread and cooled on the cover-glass bounding the area to be occupied by the hanging-drop.

The needles are made from either soft or hard glass tubing. If a brass collar is used to serve as a guide for the in-and-out movement (fig. 3), the glass tubing should be selected to fit the collar. What I use is a fraction less than  $\frac{1}{8}$  inch in outside diameter. The thicker the wall of the tubing the firmer tends to be the tip of the needle made from it. The method of making the needle is given in a paper of Barber's ('14) and in one of mine ('18). A brief account will suffice here. Acetylene or ordinary illuminating gas may be used. For a microburner use a piece of hard glass tubing bent at right angles and with the burner end closed except for the smallest aperture that will retain a flame. This may be done by heating the end and pinching it with forceps. The size of the flame may be regulated by a screw pinch-cock on the rubber tube, figure 5, h.

To make the needles, proceed as follows: 1) In an ordinary burner draw out one end of a glass tube with a capillary of about 0.3 to 0.5 mm. in diameter (fig. 5, a). 2) Lower the flame of the microburner to the smallest flame possible. Now hold the shank of the tube in the left hand and grasp the capillary at its end either with the thumb and finger of the right hand or with forceps having flat tips coated with Canada balsam. Bring the capillary over the flame and pull gently till the capillary parts. The hands should remain on the table during the

<sup>3</sup> For placing a hanging-drop after the moist chamber has been covered, a convenient pipette is one with its end drawn out into a curved capillary and the tip bent at an angle so that, on insertion into the moist chamber, the tip will touch the undersurface of the cover-slip. With a rubber tube to reach one's mouth, a small drop is readily deposited.



process and, as the capillary parts, lift the glass away from the flame by turning the hands slightly outward. The capillary will separate with a slight tug. The tip should be like that in figure 5, c. If too little heat be used and the pull made too suddenly, the capillary may part with a snap with a broken tip.

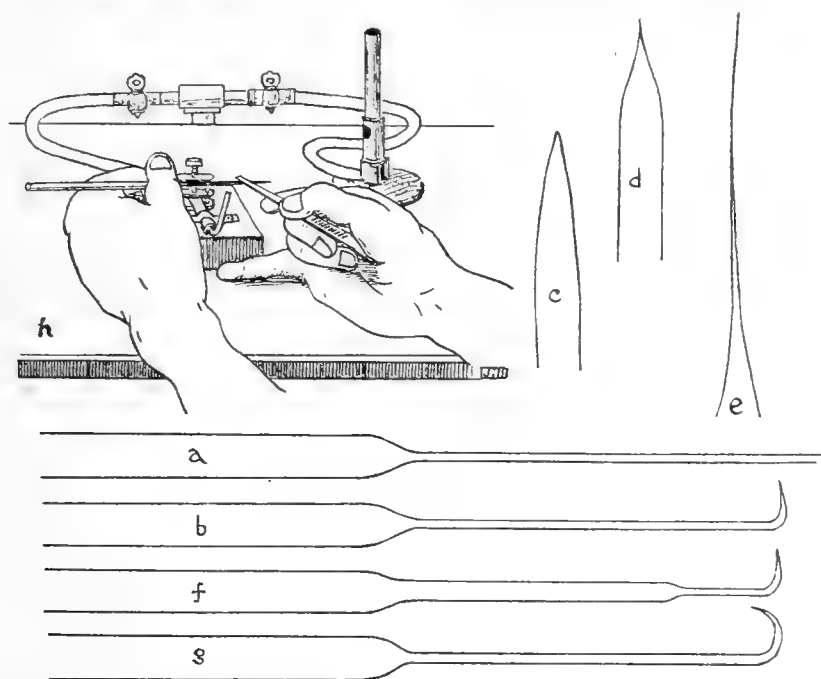


Fig. 5 Method of making the needles. *h*, position of hands when making needles over microburner; *a*, glass tube with capillary; *b*, needle with tip bent up; *c*, a good needle tip; *d*, needle tip serviceable for converting into a pipette; *e*, unserviceable tip drawn out into a hair; *f*, needle with stout shank; *g*, needle with tip bent back for cutting purposes.

If too much heat be used, the tip is drawn out into a long hair, figure 5, *e*. 3) Bend the capillary at right angles by heating it just back of the point and pushing up with a dissecting needle, 5, *b*. The length of the needle beyond the bend is conditioned by the height of the moist chamber to be used. The type of needle shown in 5, *g*, is used for cutting by bringing the upper limb of the needle below and up into the cell.

APPARATUS FOR INJECTION AND FOR THE WITHDRAWAL  
OF MATERIAL FROM A LIVING CELL

Barber's mercury pipette method, which depends upon the expansion and contraction of mercury by heat and cold, although excellent, is troublesome to make and easily broken. Taylor ('20) devised an instrument which depends upon a plunger to exert pressure on an enclosed mercury column. With mercury, however, it is difficult to maintain a plunger for any length of time without leakage. I described an apparatus ('21) in which mercury or Nujol oil is enclosed in a thin-walled steel cylinder. Pressure on the wall of the cylinder exerts the driving force necessary for injection. This works very well, but it requires special apparatus and the difficulty of securing a cylinder the walls of which are sufficiently resilient renders the apparatus somewhat unserviceable.

The apparatus shown in figure 3, does all the work of any device hitherto described and has the advantage of being extremely simple to make. All that is required is a carefully selected glass Luer syringe of about 2 cc. capacity, a piece of fine brass tubing of about 2 mm. outside diameter and two feet long (small, extra soft brass tubing used for lighting purposes is also serviceable), a metal rod 1 inch long with a hole through it large enough to receive the brass tubing, a piece of  $\frac{1}{8}$ -inch glass tubing, some de Khotinsky cement or ordinary sealing wax and an ordinary small horseshoe clamp.

First seal the metal butt of the Luer syringe to one end of the brass tubing. Slip the metal rod over the tubing and cement it an inch or two away from the syringe attachment. At the other end of the brass tubing seal a short piece of  $\frac{1}{8}$ -inch glass tubing, the free end of which has previously been drawn out into a capillary an inch or so long and about 1 mm. in inside diameter (fig. 3<sup>2</sup>).

When cementing the brass tube to the syringe attachment and to the glass tube, have a wire inserted far into the brass tube before applying the cement. The tip of the brass tube, from which the wire projects, is then coated with cement and the part to be cemented pulled over it. While the tube is still warm,

withdraw the wire with a gentle twirling motion. This draws the cement out around the ends of the brass tube on the inner surface of the projecting glass tube and prevents the formation of pockets in which air may be trapped. In the make-up of the entire system one must exercise care to prevent air from being trapped, for the presence of the air-bubbles vitiates the accurate control of pressure in the apparatus.

The brass tube where the metal rod encloses it is to be clamped to the foot of the microscope or to a base which is rigidly attached to the microscope, figure 3. The short end of the tube, projecting from the rod, is bent so that the syringe, when set into its butt stands more or less upright. The long end of the tube is carefully curved and bent, so that the glass tube which is sealed on the end will rest in the needle carrier of the micromanipulator and its capillary project over the stage of the microscope with its end about  $1\frac{1}{2}$  inches from the field of the microscope objective.

The Luer syringe must now be charged with distilled water which has been boiled and the apparatus filled to within  $\frac{1}{8}$  of an inch from the tip of the glass capillary. Before stopping, however, it is well to run water through the apparatus for some time to drive out all the air. Before charging the syringe for the last time the plunger should be coated with heavy stop-cock grease. This much of the apparatus can be kept permanently ready for use.

The micropipettes are made from microneedles drawn out of thin-walled capillary glass tubing. When finished, the shaft of the needle should be at least  $1\frac{1}{2}$  inches long and large enough to fit snugly into the glass capillary of the apparatus. This can be readily done by drawing out a supply of thin-walled glass capillaries and preserving those which fit a sample the size of the capillary of the apparatus. The needle end of the shaft should be bent at an angle, the length from the knee of the bend to the tip depending upon the height of the moist chamber. The shaft of the needle near its end is now thinly coated with de Khotinsky cement or sealing wax and, while the cement is still soft, inserted into the glass tube of the apparatus. An extra coat of cement should be added over the joint to insure the seal. The apparatus is now ready for use. The tip of the needle is brought into a

hanging-drop of water or a solution to be injected and converted into a pipette by jamming the tip against the under surface of the cover-slip until it breaks off. During the process continual pressure should be exerted on the plunger of the syringe in order to prevent pieces of glass from being sucked into the pipette. Occasionally, while attempting to make the needle in the flame, a serviceable pipette results instead. When the pipette is finally in place, all or most of the air in it should be driven out.

One can readily see that the sealing of the micropipette into the apparatus must be done away from the microscope. It is in this operation that the type of micromanipulator fastened on a pillar is of advantage. The pipette has to be frequently changed, and it is very convenient to be able to release the microscope from its base by loosening its clamps and to slip it out of the way. As soon as a fresh needle has been inserted, the microscope is readily slid back into place. For this purpose the base on which the microscope rests is provided with guides to insure its true return. When exchanging a pipette, care must be taken not to clog the lumen. This can be done by using a minimum amount of cement and by having the lumen of the tube into which the shaft of the pipette is to be inserted as clean as possible.

The use of thin-walled tubing for making the micropipette is to insure having the largest bore possible at the tip of the pipette. The thickness of the wall and the size of the lumen of the glass tube tend to maintain their original proportions when drawn out in a flame. Often, however, it is more convenient to have pipettes with stouter walls. Such pipettes are less readily broken but, owing to the smaller-sized lumen, run the risk of quickly clogging. The best pipettes are made from hollow needles with a rapidly tapering tip (fig. 5, d), for needles with a long taper are apt to break anywhere.

A necessary precaution is to have the capillary from which the needle is to be made perfectly dry. The presence of the least moisture may result in alternating columns of water and air in the pipette tip which no amount of pressure will expel.

Water seems to be the best medium for transmitting pressure in the apparatus. Mercury is apt to break and allow air or

water to leak past it when it reaches the tip of the pipette. When this occurs, the separated droplet of mercury clogs the aperture. Mercury also tends to leak past the best plunger made.<sup>4</sup> The disadvantage of using water is the risk of its diffusion into the solution to be injected. If a considerable amount of the solution be drawn into the pipette, this risk is minimized. A good method is to color the water (e.g., with Nile-blue chlorhydrate or with neutral red). The solution drawn into the pipette from a hanging-drop is then visible by contrast. For ordinary purposes a cushion of air between the water and the injection fluid serves well.

Oil is unsuitable because, in spite of all precautions, it occasionally comes into contact with the hanging-drop containing the tissue to be operated upon; it then spreads over the surface of the drop and injures the preparation. It also dissolves de Khotinsky cement and sealing wax which are so convenient for cementing the pipette to the apparatus.

Manipulation of the syringe is facilitated by fastening it in a frame and by using a milled screw to press the plunger. I use a microscope for this purpose with the objective, substage and mirror removed. The syringe is passed through the center of the microscope stage where it is held firmly with a tight-fitting collar of cork. The lower end of the microscope tube rests on the top of the plunger so that pressure can be brought to bear on it by either the coarse or fine adjustments. There is no need of fastening the plunger to the microscope tube, because the resiliency of the water in the apparatus is sufficient to cause suction in the micropipette when the plunger is released from pressure.

#### APPENDIX

Barber's instrument is based on the principle of a carrier pushed along a groove by a screw at one end. By having a series of three carriers built up on one another, each traveling in a different direction, movements in any one of three dimensions may

<sup>4</sup> Leakage in the syringe can be avoided by placing a cushion of oil between the plunger and the mercury. This may also be done when water is used.

be imparted to a needle clamped to the top carrier. Hecker ('16) improved Barber's instrument, but added materially to the intricacy of its make up.

Other investigators that I know of who have devised instruments for micro-operative work are Schmidt ('69, '70), Birge ('82), Chabry ('87), Schouten ('05, '11), Tchahotine ('12, '21), McClendon ('07), Malone ('18), Bishop and Tharaldsen ('21).

Schmidt's instrument is one of historic interest only. I have already described it ('18). Chabry used a delicate spring device with which he could shoot the tip of a glass needle into an ovum to any desired depth. Schouten uses his for the isolation of bacteria. It consists of a pillar carrying a needle which may be mechanically raised and lowered. For the horizontal movements Schouten depends upon pushing the microscope on a base. McClendon attached an up-and-down movement to a Spencer mechanical stage. Tchahotine uses a mechanism attached to the tube of his microscope from which extends a glass needle curved in such a way as to bring its tip into the field of a low-power objective where it is brought into focus. Dissection of cells is carried out by moving the microscope tube and by pushing the cells against the needle tip by means of the mechanical stage of the microscope. Malone uses Schouten's method, but, instead of having a special pillar with a raising device, he mounts his pipette carrier on the tube of a second microscope whose adjustments serve as a means for raising and lowering the pipette. Bishop and Tharaldsen have a simple instrument based on a principle somewhat resembling mine but lacking in proper control for one of the two lateral movements. Recently I have heard that Zeiss is manufacturing a micro-dissection instrument which is a modification of Barber's apparatus with both coarse and fine adjustments.

Tchahotine and Bovie have recently devised a method for producing localized injury in a cell by means of ultra violet rays. The method is very ingenious but, of course, is rather limited in its application to micro-dissection.

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Resumen por los autores, Carl Hartman y Chester H. Heuser.

Un fondo negro para la fotografía de objetos en medio líquido.

Los autores dan a conocer un método para obtener un fondo sólido de color negro en la fotografía de objeto delicados y translúcidos, colocados en agua ó solución salina. Los objetos se colocan en una vasija de tamaño apropiado y esta á su vez se sitúa sobre un trozo de vidrio negro, que debe sumergirse en agua, pues para el éxito del método es necesario evitar los espacios con aire entre el objeto y el fondo. Los objetos muy pequeños se colocarán en un vidrio de reloj de vidrio transparente y este se coloca en un vidrio de reloj negro bleno de agua.

El movimiento de la superficie del agua sobre el objeto que ha de fotografiarse se impide por medio de una placa de vidrio ó cubreobjetos que se desliza sobre la vasija que contiene al objeto cuando aquella está por completo llena de líquido. Para la fotografía se usa iluminación lateral. En la lámina que acompaña al presente trabajo se han representado ejemplos de fotografías tomadas con este método.

Translation by José F. Nonidez  
Cornell Medical College, New York



## A BLACK BACKGROUND FOR PHOTOGRAPHING OBJECTS IN A LIQUID MEDIUM

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ONE PLATE (SEVEN FIGURES)

While connected with The Wistar Institute of Anatomy, under whose auspices embryological work on the opossum was done several years ago, the writers used with success the device described below for securing solid black backgrounds in the photographing of opossum eggs. The description is here given in the belief that it may be of use to others who wish to photograph similar delicate and translucent objects in a liquid medium.

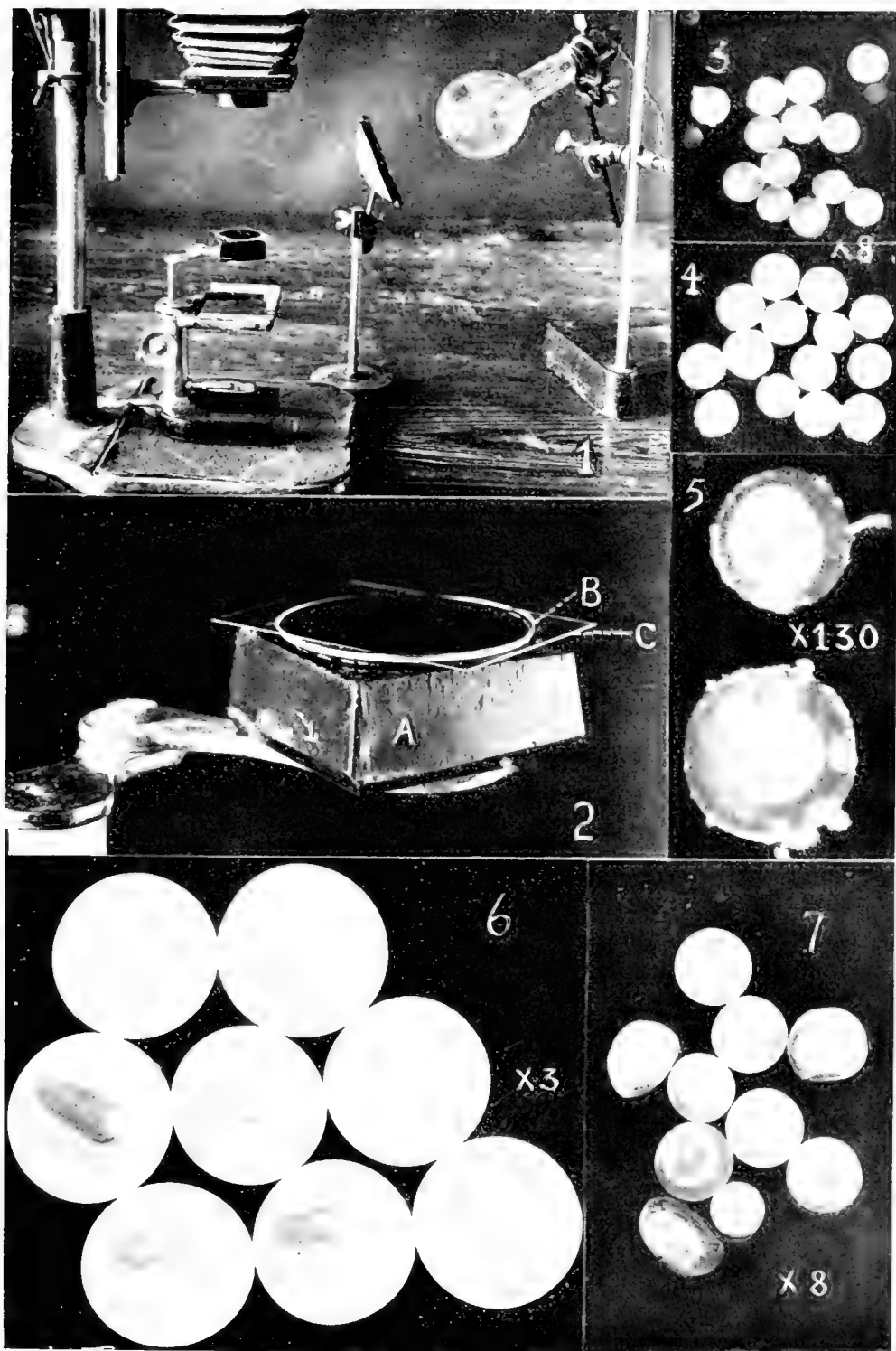
The method depends for its success upon the fact that reflecting surfaces are reduced to a minimum, air-spaces between the container and background being strictly avoided.

For photographing very small objects the arrangement shown in figure 2 was used. *A* is a dish made of black glass, known commercially as an embryological watch-glass; it has a rather deep concavity, which is filled with water. Into this is placed the shallow clear watch-glass, *B*, filled to the brim with physiological salt solution and containing the objects to be photographed. To form a stable surface the cover-glass, *C*, is slipped over the salt solution. Care should be taken to avoid all air-bubbles. The background is now a solid black. Unilateral illumination, shown in figure 1, may be used. Several samples of eggs photographed in this way are shown in figures 3 to 7.

Figure 6 reproduces a batch of embryos which were too large to photograph in the shallow watch-glass as detailed above. Hence the watch-glass was placed in a shallow Stender dish of salt solution, where, by virtue of its concavity, it functioned merely to keep the eggs together. A sheet of black glass served as a background; but here again water was necessary to dis-

place the air between the Stender dish and glass plate. This was accomplished by laying the glass plate in the bottom of a larger dish, covering it with water, and setting the Stender dish containing the specimens into it, air-bubbles being avoided as before.

The black glass plate set in water has been successfully employed by us as a black background for various objects that have to be photographed in a liquid medium.



Resumen por la autora, Ethel L. Anderson

El desarrollo de los derivados faringeos de la vaca (*Bos taurus*)

El presente trabajo se basa en el estudio de la farínge de ventiocho embriones de vaca, de 3,5 á 163 mm. de longitud. Las conclusiones derivadas de este estudio son las siguientes: 1) La segunda bolsa se abre normalmente en la vaca; la cuarta no llega á tocar al ectodermo. 2) La tiroides media experimenta una evolución típica. 3) El timo III, de origen exclusivamente endodérmico se divide en una cabeza, un cordón intermedio, un segmento medio-cervical, un cordón cervico-torácico y un segmento torácico. De estas partes los cordones cervico-torácicos e intermedios presentan transformaciones típicas tan solo en los estados avanzados. 4) Las paratiroides aparecen en forma de proliferaciones del epitelio de las bolsas, adyacente á los arcos aórticos correspondientes, y están muy vascularizadas desde sus comienzos. 5) La paratiroides III participa ligeramente en el 'descenso del corazón' y no está asociada con la tiroides como una paratiroides interna, sino que permanece junto á la bifurcación de la carótida, situada medialmente á dicho vaso. 6) La paratiroides IV viene á alojarse en el aspecto medio-caudal del lóbulo lateral de la tiroides, pero nunca está incrustada en la glándula. 7) El cuerpo últimobranquial puede distinguirse todavía del material de la tiroides en el embrión de edad más avanzada de los examinados. 8) El timo IV es solamente una estructura transitoria, que no puede reconocerse después del estado de 14 mm. Solamente en dos casos de los embriones más avanzados existía un timo accesorio, el cual pueda tal vez considerarse como el timo IV.

Translation by José F. Nonidez  
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THE DEVELOPMENT OF THE PHARYNGEAL DERIVATIVES IN THE CALF (BOS TAURUS)

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ONE PLATE (FIVE FIGURES)

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INTRODUCTION

Despite the vast amount of work on the pharyngeal derivatives in many animals, literature shows but few references to these bodies in the calf. Verdun has given a short description of the pharyngeal derivatives as found in one embryo, 160 mm. in length, together with a reference to a description of a smaller embryo by Woelfler, whose paper, however, is not accessible to the present writer. Groschufi ('96) worked with considerable accuracy on several animals, including the calf, and he has been quoted by a number of authors, among whom are Maurer and Simon. This latter author, whose chief interest was in the fate of the so-called 'lateral thyroid' (L'ebauche lateral de la glande thyroide), included in the material which he studied more than twenty calf embryos, none of which were under 20 mm. in length.

It is the purpose of this paper to present the early development of the pharynx of the calf with reference particularly to the pharyngeal derivatives. In the parathyroids especially one

can see a peculiarity in development which is apparently quite different from other forms. But brief reference will be made to the general literature; for a detailed review of the early research the reader is referred to the review by Verdun ('98) as well as to the papers of Grosser ('11), Hammar ('11), and Camp ('17).

A series of calf embryos, the property of the Department of Histology and Embryology of Cornell University, was placed at my disposal. It consisted of twenty embryos, ranging from 3.5 to 50 mm. in length and cut in transection, and the heads of eight older embryos, 58 to 163 mm. long, which were cut in sagittal section. Twelve frontal or sagittal series of younger embryos were also employed, chiefly for orientation.

### *The median thyroid*

The development of the median thyroid is essentially that described for other mammals. The youngest embryo of the series (3.5 mm. estimated length) shows four branchial pouches, only three of which have an ectodermal contact; the fourth pouch was not observed to reach the ectoderm in any of the embryos which were examined. The 'median thyroid' appears in this embryo as a hollow bud from the floor of the pharynx, in the region just posterior to the first pouch. With the rupture of the 'thyreoglossal duct' (8.5 mm. embryo) the thyroid is left as a short solid cord or plate of cells, morphologically, as at the time of its origin, cephalodorsal to the truncus aorticus. A slight depression on the floor of the pharynx, the foramen caecum, marks the upper extremity of the duct and the point of origin of the gland. The 8.5-mm. embryo was the only one in which the foramen caecum was evident. The lower extremity of the duct, the 'ductus thyreoideus,' extends about 0.12 mm. above the body of the gland.

At about this age (8 to 11 mm.) the lateral expansion of the thyroid is dependent apparently on the growth tensions in the individual embryo, and presents considerable variation. But in every case it has maintained its position on the cephalodorsal side of the truncus aorticus. The ductus thyreoideus can be

traced forward sometimes as much as 0.3 mm., depending on the length of the downward shifting and somewhat on the angle of the section. Though the base of the 'duct' is moderately thick, the upper part dwindles down to no more than two or three cells to the section.

In their expansion the lateral lobes come into close contact, topographically, with the derivatives of the caudal pharyngeal complex—the ultimobranchial body and parathyroid IV. In the 14-mm. embryo the ultimobranchial body lies medial to and close up against the lateral lobe of the thyroid, and in the early stages, when the ultimobranchial body is large, it constitutes nearly the entire lateral lobe, while the thyroid itself is represented by slender cords or plates of cells, loosely strung together by connective tissue, as shown in figures 2 and 4; the median portion of the thyroid is more compact. Parathyroid IV lies median to the dorsal aspect of the lateral lobe, entirely outside of the parenchyma of the gland. As the embryo grows the parathyroid and the ultimobranchial body sink lower in the lateral lobe and the thyroid material comes to nearly surround the latter.

Though in earlier embryos the derivatives of the caudal pharyngeal complex lie in or near the cephalic end of the lateral lobe, in those of 30 mm. and over they are found well down within the body of the lobe; parathyroid IV is sometimes visible even after the sections have passed below the level of the thyroid itself. This shifting in position is due partly to the upward expansion and growth of the lateral lobe and partly to the continued 'descent' of the other bodies. In the 50-mm. embryo the lateral lobes contain the characteristic dark patches of cells (cf. fig. 3), the cords of the ultimobranchial body. There is, as yet, no loosening of the cells of the thyroid cords to form vesicles and no secretion of colloid until the embryo has reached 140 mm. in length. Simon ('96) also described the appearance of colloid at this time.

### *Complex III*

In the calf embryos one finds four well-formed pouches. Only the first three of these were ever observed to establish an ecto-

dermal contact; the fourth pouch was never observed to reach the ectoderm in the series of embryos which was studied. The 8-mm. embryo showed the rupture of the membrane separating the second pouch from the exterior; this condition is quite common in calf embryos (and occasionally observed in human); in some instances it may be due to rough handling, but as the rupture was observed in several embryos which were well preserved and showed good morphological relations it was concluded to be a normal occurrence. The first of the aortic arches—which form on the anterior aspect of each pouch—has already broken its connection with the dorsal aorta in the 3.5-mm. embryo; the second, though small, is still intact; the third is large and fills most of the space in the third branchial arch, and the fourth and sixth aortic arches are not yet formed. In the 8-mm. embryo the forward growth of the anterior pouches has broken the second as well as the first arch; the remaining three arches (third, fourth, and sixth) are well formed.

The 8.5-mm. embryo is the youngest in which there is to be noted from the medial border of the third pouch the ventral elongation which is to form the entodermal thymus. As the embryo develops (9.5 to 10 mm.) thymus III becomes elongated, extending down from the pouch, directed ventrally and medially and pointed toward the truncus aorticus. In the more cephalic sections it is seen in the form of a hollow tube, dorsal and a little external to the third aortic arch (the primitive carotid). It follows the line of the carotid, swinging to the lateral and finally to the ventral side of that vessel.

When the embryo is 14 mm. in length the thymus and parathyroid III have lost their connection with the pharynx. The head of the thymus is lodged in the caudal and dorsal part of parathyroid III and the gland extends back from this as a slender cord with the lumen nearly obliterated, and follows roughly, as before, the line of the carotid. In the thoracic region the two sides of the thymus make a rather marked medial turn and come to lie over the pericardial cavity, a little to the left of the mid-line. Here the walls of the thymus become enlarged and lobulated; the branching cords from either side are apparently fused along their region of contact.



In connection with the development of the thymus it may be stated that a cervical vesicle is formed essentially as in man and possesses the same relations to the ganglion nodosum. It forms no part of the thymus, as it does in the pig.

In the 30-mm. embryo the division of the thymic cord and body into segments is definitely noted for the first time. The head of the thymus, slightly enlarged, lies, as before, in connection with the lower pole of parathyroid III and the whole group has shifted a little to the median side of the carotid artery, on its dorsal aspect. From the head extends a thin cord of cells, called by Badertscher ('15, who adopted the terminology of Kastschenko, '87) the 'intermediary cord,' which connects the thymic head with the 'mid-cervical segment.' In the region of the thyroid the 'intermediary cord' is very small and lies ventral to, and a little anterior to the brachiocephalic vein which is formed by the union of the subclavian and jugular veins. Below this, in the region of the truncus proper and also ventral to the superior vena cava, the thymic cord diminishes markedly in caliber to form the 'cervicothoracic cord' which connects the 'midcervical' with the 'thoracic segment.' The thoracic segment lies in the upper section of the thorax, is expanded in contact with the pericardium, and is markedly lobulated.

The above description will hold, in the main, until the 80-mm. stage is reached. Only the following differences need be noted. In the 39-mm. embryo there is no enlargement in the midcervical region. The 40-mm. stage shows a shifting of parathyroid III so that it (and consequently the head of the thymus) lies below the bifurcation of the carotid and well medial to that vessel.

After the 80-mm. stage the head of the thymus becomes markedly enlarged and lengthened. At 110 mm. the intermediary cord begins to enlarge, and at 130 mm. shows slight lobulation. Complete older series would undoubtedly show a transformation of the entire thymic cord as in the pig (Badertscher).

The dorsal element of complex III, parathyroid III, appears for the first time in the 9.5 to 10-mm. embryo in which the thymus has already elongated. It lies on the anterior and dorsal surface of the third pouch, and extends well from the pharynx

to the free margin of the pouch. Histologically, it is composed, from its earliest appearance, of cords of epithelial cells, proliferated by the entoderm of the pouch and associated from the start with a rich network of fine capillaries.

The question of the vascularization of the parathyroids is an important one and involves possibly the question of the cause of their appearance. In the human embryo they appear as rather thick, but definitely limited proliferations of epithelium on the dorsal side of the third and fourth pouches, and it is not until late in their history that they are broken by vascular channels. In the calf, on the other hand, blood vessels appear along the anterior side of the third pouch before the epithelial proliferation is even noted (9 mm. Sag.) and, after the proliferation, there is a subepithelial plexus of blood vessels not only between the cords of cells, but also to the outside of the gland. The connection of this vascular network appears to be with the anterior side of the third aortic arch, and with the dorsal side of the external carotid, going to parathyroid III on either side of the common carotid; a few of the younger embryos show direct vascular connections between the posterior side of the carotid and the parathyroid (fig. 5), and still others show combinations of these sources. In all cases the third aortic arch lies along the anterior and dorsal surface of the third pouch and is unquestionably the source of some of the blood for the parathyroid.

In the early embryos parathyroid III lies dorsal to the common carotid artery, between it and the anterior surface of the pouch, and the surface of the parathyroid is slightly concave to conform to the shape of the carotid about which it fits. Topographically it is anterior to the bifurcation of the carotid.

In the 14-mm. embryo, in which the derivatives have broken from the pharynx, the parathyroid has the shape and position which characterize it for a number of stages; it is in the form of a thick crescent, slightly elongated in the vertical plane. On its anterior side it is concave toward the carotid and forms a cap around that vessel, just cephalad of the bifurcation of the carotid. It lies a trifle lower than the carotid body, which is seen here for the first time. The horns of the crescentic parathyroid seem

to be shaped about the sides of the carotid by the hypoglossal nerve (XII) on the exterior, and the superior laryngeal nerve on the interior surface. In the younger embryos the hypoglossal nerve lay entirely caudal and ventral to the third complex, lateral to the fourth complex and its aortic arch. A slight descent of the head of the third complex together with the forward growth the more lateral material is responsible for the altered relations. The head of the thymus is lodged in the lower pole of parathyroid III.

Subsequent stages (30 mm. and up) see a backward shifting of parathyroid III, so that most of its body now lies caudal to the bifurcation of the carotid and hence posterior to the carotid body. It does not, however, come into contact with the lobes of the thyroid as does the other parathyroid. In its shifting it comes to lie well medial to the carotid and offers a flat or slightly concave surface to that vessel. Numerous small blood vessels are seen which enter parathyroid III from the carotid both directly (fig. 5) and out through the carotid body.

#### *The caudal pharyngeal complex*

The caudal pharyngeal complex—to include under a common heading, the derivatives parathyroid IV, the ultimobranchial body, and thymus IV, of the more caudal pouches—has been a frequent source of discussion among writers, both from the standpoint of origin and from the standpoint of fate. Especially is this true of the ultimobranchial body (the 'lateral thyroid,' 'post-branchial body,' 'suprapericardial body' of other writers).

Briefly, the morphology of the fourth or caudal pharyngeal complex as it appears in successive stages in the calf embryos of the series studied is as follows:

The fourth pouch, as stated above, does not reach the ectoderm, but lies in the posterior region of the pharynx caudal to the third pouch and, because of its tardy development, is smaller than and median to the wings of the third pouch. In the 8.5-mm. embryo a long posterior and a short anterior diverticulum are evident on the ventral side of the complex and the fourth aortic arch forms to the lateral side of the pouch. The anterior

diverticulum elongates (9.5 to 10 mm.) to form the so-called 'thymus IV' with its axis directed toward the truncus as in the case of thymus III. The posterior diverticulum forms the ultimobranchial body, and in the angle between it and thymus IV, on the lateral side of the pouch in the region toward the fourth aortic arch is parathyroid IV. Like parathyroid III, parathyroid IV is from the first an epithelial proliferation from the wall of the pouch and is supplied with a rich subepithelial network of blood channels. But here it has been impossible to determine any definite and constant connection of the vascular network. In the 11-mm. embryo there is one direct blood vessel from the posterior side of the fourth aortic arch to parathyroid IV. The ultimobranchial body and thymus IV have a common opening into the pharynx, but their relative size soon changes so that thymus IV (14.5 mm.) appears to be an appendage of the ultimobranchial body; parathyroid IV, a vascularized proliferation, lies between them on the dorsolateral aspect of the pouch.

The ductus pharyngeo-branchialis IV, which early connects the complex with the pharynx, has ruptured and entirely disappeared in the 14-mm. embryo; thymus IV, nicely shown in sagittal section in the 14-mm. stage, has disappeared as such, and of the complex there only remain parathyroid IV and the ultimobranchial body. These come to lie close to the upper pole of the lateral lobes of the thyroid as that gland develops. Parathyroid IV is the more dorsal and medial; the ultimobranchial body is fast against the inner surface of the lateral lobe, but separated from the thyroid by a distinct connective-tissue sheath. A cord of cells connects the anterior side of the ultimobranchial with the parathyroid, and in the parathyroid, at the head of this cord, is a cavity—the remains of the cavity of the fourth pouch.

Even at 15 mm. the ultimobranchial body shows, in its scalloped edge, the tendency to 'bud,' which has led some authors to the belief that this, the 'lateral thyroid,' after its inclusion in the lateral lobe of the thyroid, is transformed into a part of the thyroid gland.

While the relative size and position of parathyroid IV and the ultimobranchial body vary in embryos 20 mm. to 50 mm. in

length, the same general relations hold. Parathyroid IV is round or oval in form, always situated in the dorsomedial aspect of the lateral lobe of the thyroid, sometimes above and sometimes below the level of that gland. Often, especially in the later stages, the parathyroid will lie low on the lateral lobe. Some of the blood enters it directly from the dorsal side of the carotid artery (24 mm.), while in other cases (40 mm., 50 mm.) the parathyroid is supplied with a branch from the accessory thyroid artery. It always contains, on its ventral side, a cavity from which a solid cord of cells runs forward to join with the ultimobranchial body (fig. 4). This latter structure is embedded more or less intimately in the lateral lobe of the thyroid and shows in the early stages a heavy wall and a narrow, crescent-shaped cavity. About it the cords of the thyroid tend to be thin and separated by connective tissue; below the level of the ultimobranchial body the material of the thyroid is more closely packed. Gradually the thick wall of the ultimobranchial body loosens and sends out buds which shape themselves into narrow cords or plates of cells, intermingling with those of the thyroid proper. Though in form resembling the material of the thyroid, histologically they are quite different. The nuclei of the epithelium of the thyroid are of varying size and shape and contain numerous small bits of chromatin, peripherally placed. The nuclei of the cells of the ultimobranchial body, on the other hand, are smaller, rounder, more nearly of a size, and more closely packed; they contain two or three large nucleoli, centrally located. Even in the 50-mm. embryo, in which the sections are rather thick, the material of the ultimobranchial bodies can be easily distinguished in the midst of the substance of the thyroid by its darker staining qualities. Figure 3, of a 33-mm. embryo, may be compared.

From this time on, with the marked increase in the size of the thyroid, the ultimobranchial body becomes relatively smaller and smaller and shows as thick branching cords of darkly staining cells at the dorsal side of the lateral lobe of the thyroid and at the 'hilus,' around the entrance of the blood vessels. These cords of cells branch out, but do not join with the parenchyma of the thyroid.

In the 40-mm. embryo, in connection with the lower pole of the parathyroid, is found an instance of an accessory thymus (IV). Its appearance is that of the thymus (III) and its relation to the posterior pharyngeal complex would suggest that it represents a thymus IV. A calf of 130-mm. length shows a similar structure.

#### SUMMARY

The following points have been noted in the morphology of the pharyngeal derivatives in the calf:

1. The median thyroid undergoes a typical evolution.
2. Thymus III, of entodermal origin only, divides into head, intermediary cord, midcervical segment, cervicothoracic cord, and thoracic segment.
3. Of these, the intermediary and cervicothoracic cords show thymic transformation only in the older stages.
4. The parathyroids appear as proliferations of the epithelium of the pouches, adjacent to the corresponding aortic arches, and are highly vascular from the first.
5. Parathyroid III is but little involved in the descent of the heart; it is not associated with the thyroid as an external parathyroid, but remains near the bifurcation of the carotid and medial to that vessel.
6. Parathyroid IV becomes lodged on the mediocaudal aspect of the lateral lobe of the thyroid, but is never embedded in that gland. It is connected, in all the embryos examined, by a cord of cells to the ultimobranchial body.
7. The ultimobranchial body can still be clearly distinguished from the material of the thyroid in the oldest embryo examined.
8. 'Thymus IV' is but a transitory structure, not recognizable after the 14-mm. stage.
9. In only two cases in the older embryos was an accessory thymus noted which might possibly be considered as 'thymus IV.'
10. From the evidence it may be concluded that: a) The second pouch normally opens in the calf; b) the fourth pouch fails to reach the ectoderm.

I wish to take this opportunity to thank Dr. B. F. Kingsbury for the material he has put at my disposal and for helpful criticisms and suggestions. I am also indebted to Prof. S. H. Gage for the material which he has loaned to me.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Section through the pharynx, showing the third branchial pouch and the formation of parathyroid III on the anterior and (dorsal) side of the pouch. Section also shows direct vascular connection from the posterior side of the third aortic arch to the anterior surface of the corresponding pouch. The second pouch is open. Embryo calf, 11.5 mm. Photograph,  $\times 33\frac{1}{3}$ .

2 Section through the caudal pharyngeal complex, to show the characteristic relations. Parathyroid IV, on the right, shows the characteristic cavity at the head of the cord which connects the parathyroid with the ultimobranchial. Embryo calf, 14 mm. Photograph,  $\times 33\frac{1}{3}$ .

3 Section through the lateral lobe of the thyroid in a more advanced stage. The cords of the ultimobranchial body are clearly distinguishable. Embryo calf, 33 mm. in length. Photograph,  $\times 33\frac{1}{3}$ .

4 A section through the caudal pharyngeal complex, to show the connection of the ultimobranchial body to the parathyroid IV by a cell cord. Compare with figure 2. Calf embryo of 20-mm. length. Photograph,  $\times 33\frac{1}{3}$ .

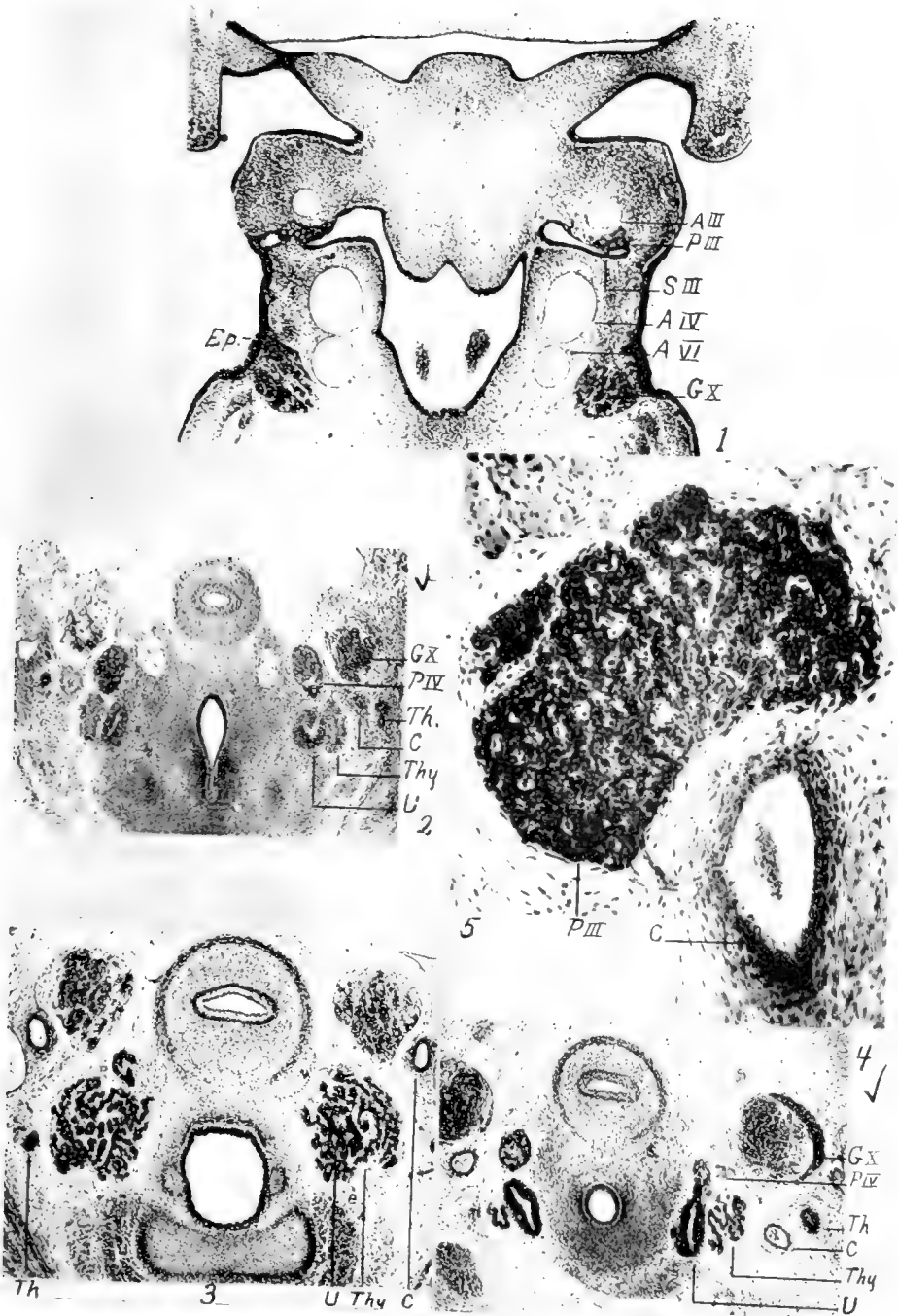
5 Section through parathyroid III and the carotid artery, to show the direct vascular connections between the two. Embryo calf, 30-mm. length. Photograph,  $\times 125$ .

### ABBREVIATIONS

*A. III*, third aortic arch  
*A. IV*, fourth aortic arch  
*A. VI*, sixth aortic arch  
*C.*, carotid artery  
*Ep.*, epibranchial placode  
*G. X.*, ganglion nodosum (X)  
*P. III*, parathyroid III

*P. IV*, parathyroid IV  
*S. III*, sacculus branchialis III (pouch III)  
*T. A.*, truncus aorticus  
*Th.*, thymus  
*Thy.*, thyroid  
*U.*, corpus ultimobranchiale







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Resumen por el autor, Alfred S. Romer.

La comparación de los coracoides de los mamíferos y reptiles.

Para establecer las homologías de los elementos del coracoides de los mamíferos con los del de los reptiles, debe compararse la cintura escapular de los monotremas con la de los antepasados terápsidos de los mamíferos, y no con la de los reptiles modernos que no están relacionados con ellos. Los argumentos de Gregory y Hanson para demostrar que el coracoides y epicoracoides de los monotremas son homólogos con el 'coracoides' y 'epicoracoides,' respectivamente, de los reptiles vivientes, no son suficientes para demostrar este punto.

Cuando se consideran los coracoides de los mamíferos y reptiles en su verdadero orden filético, conforme han hecho Broom y Watson, puede verse que el coracoides de los mamíferos es el elemento primitivo posterior, que falta en los reptiles modernos. El 'coracoides' de estos últimos es el elemento primitivo anterior, homólogo al epicoracoides de los monotremas, faltando en los mamíferos superiores.

Translation by José F. Nonidez  
Cornell Medical College, New York

## THE COMPARISON OF MAMMALIAN AND REPTILIAN CORACOIDS

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### ONE FIGURE

The problem of the homologies of mammalian and modern reptilian coracoids has been complicated by the palaeontological discoveries of the past twenty years, especially those of Broom, Case, Watson, and Williston which show that most of the extinct primitive reptiles and all of the extinct mammal-like forms (therapsids) possessed two coracoid elements.

Williston, in 1911, demonstrated that the modern reptilian 'coracoid' corresponds to the anterior of the two primitive elements, and this interpretation is generally accepted. No agreement, however, has been reached on the question of the derivation of the mammalian coracoid.

In 1912 Broom compared the mammalian coracoid with that of the therapsid ancestors of the mammals, and concluded that it corresponds to the posterior coracoid of the more primitive forms and is not homologous, therefore, with the 'coracoid' of existing reptiles. In 1917 Watson traced the two primitive coracoids through a graded phyletic series of mammal-like reptiles to the two coracoid ossifications of the monotreme, and confirmed Broom's results. Gregory, on the other hand, compared the monotreme girdle with that of lizards and *Sphenodon*, and in 1918 reached the opposite conclusion, namely, that the coracoid of mammals corresponds to that of living reptiles—the anterior primitive element. He suggested also that the epicoracoid bone of monotremes may correspond to the 'epicoracoid' cartilage of lizards and *Sphenodon* and to a similar cartilage which is generally believed to have existed in a num-

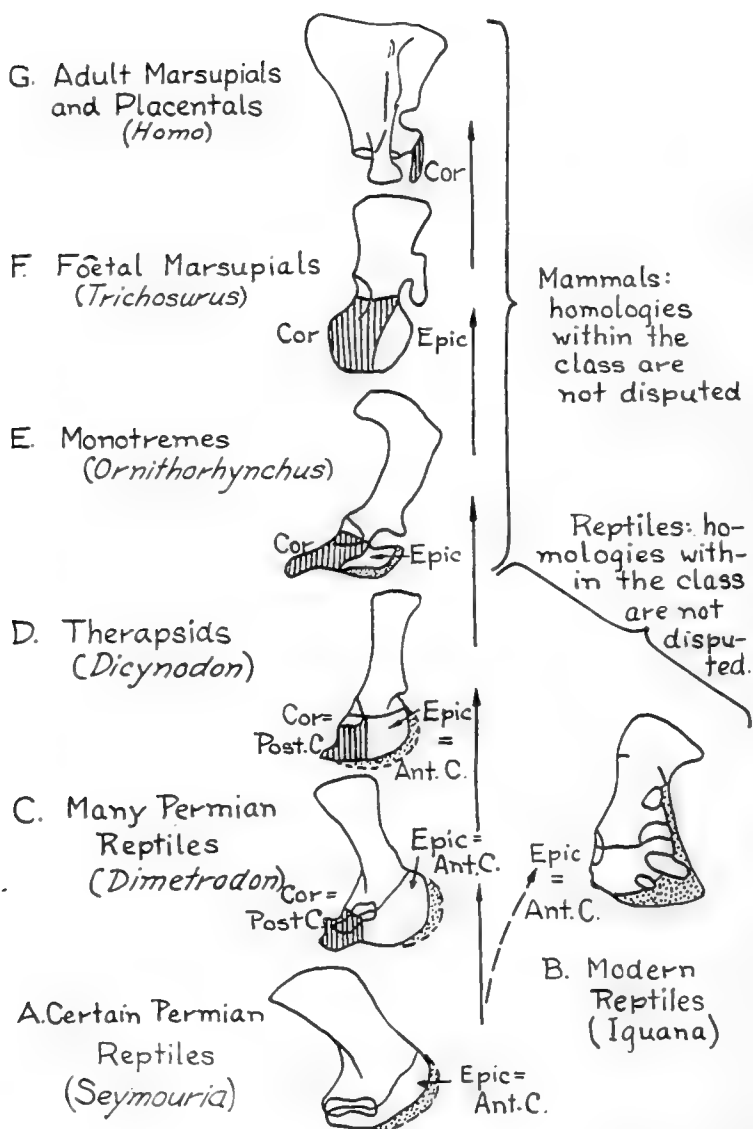


Fig. 1 Primary shoulder-girdles of representative mammals and reptiles, arranged in proper genetic sequence. The mammalian coracoid and its probable homologue, the reptilian posterior coracoid, are hatched. The epicoracoid and anterior (reptilian) coracoid are unshaded. The 'epicoracoid' cartilage and other peripheral cartilage are stippled. (F after Broom.)

ber of extinct reptiles. He notes that Watson's paper was received too late for consideration.

In 1920 Hanson restated Gregory's argument, without adding any new element to the discussion. Additional citations were made to show the probable presence of an 'epicoracoid' cartilage in extinct forms. The monotreme girdle was again compared with that of the lizards and *Sphenodon*. Watson's work was mentioned, but not considered, and, although three years had elapsed, no comparison was made between the girdle of the monotreme and that of its therapsid ancestors.

All are agreed as to the homologies of the coracoids within the limits of the class Reptilia. A few of the most primitive Permian forms (as *Seymouria*) have but one element, the anterior (fig. 1). Most other primitive fossil reptiles have in addition a second element which carries the posterior half of the glenoid cavity. From these primitive types the modern reptilian coracoid is derived, either through the secondary loss of the posterior element or through the retention of the most primitive condition of the girdle in which the posterior coracoid had not yet made its appearance. The mammal-like reptiles, on the other hand, possess two coracoids which are clearly homologous with those of their primitive ancestors. In this type of girdle the anterior element is almost completely excluded from the glenoid cavity by the posterior one (Watson).

Within the class Mammalia, the homologies are unquestioned also. The coracoid of the adult marsupial is clearly that of the placental. In the embryo, as noted by Broom ('97, '99, '02, '12) and confirmed by Watson ('17) and by Hanson ('20), it extends from the glenoid cavity to the midline. The condition of the coracoid of the embryonic marsupial is repeated in the coracoid of the adult monotreme. The epicoracoid of the latter is represented, according to Broom, by a membrane anterior to the coracoid of the embryonic marsupial.

It remains, then, to compare the two classes. It is in the selection of the proper forms between which to make the comparison, that the workers belonging to the two groups disagree. The monotreme is taken by all as representing the most primi-

tive mammalian condition. Broom and Watson compare the monotreme with the therapsids, from which it is generally agreed that the mammals have been derived. The therapsids possess two coracoid elements, of which the posterior one occupies the lower half of the glenoid cavity and extends downward and backward from it. This is the position occupied by the true coracoid of monotremes. Anteriorly, in the therapsid, is the primitive anterior coracoid element, and the epicoracoid is anteriorly placed in the monotreme. The anterior coracoid of the therapsid and the epicoracoid of the monotreme occupy essentially the same position, and both are excluded from the glenoid. The only noticeable difference lies in the appearance of a gap in the upper edge of the monotreme epicoracoid, which does not reach the scapula above as does the therapsid anterior coracoid. The appearance of this gap has been explained by Watson upon functional grounds.

Both Gregory and Hanson compare the coracoid of the monotreme with that of *Sphenodon* and the lizards. Hanson states that 'genetic connection and homology' exist between them. This 'genetic connection,' of course, would not apply to any phyletic relationship between the groups of animals involved. There is no evidence, other than the suggested homology of the coracoids, which would tend to show that either *Sphenodon* or the lizards, which belong to two widely divergent subclasses of reptiles, are in any way ancestral to mammals. On the other hand, almost all students of mammalian phylogeny, such as Owen, Seeley, Broom, Gregory, and Watson, are agreed that the therapsids do occupy such a position.

The two modern reptilian groups mentioned and the ancestors of the mammals have followed separate lines of development since the Permian at least. Admittedly the shoulder-girdle of both the modern reptile and the monotreme has been greatly modified from the common primitive type. It is difficult to understand how a 'genetic connection' is possible between the shoulder-girdles of what appear to be end forms of divergent lines. The common meeting-point for the establishment of a genetic connection between mammals and modern reptiles is



the primitive reptile of the Permian, and it is generally accepted that this common ground is to be reached from the mammalian side only through the therapsids.

Three main lines of argument are followed by Gregory and Hanson in making the comparison: 1) The epicoracoid of monotremes is held to be the homologue of the 'epicoracoid' cartilage of lizards and *Sphenodon* and of a cartilaginous area which existed anterior to the anterior coracoid of some primitive and mammal-like reptiles. 2) The muscular origins on the two coracoid bones of monotremes are similar to those on the coracoid and 'epicoracoid' of lizards and *Sphenodon*. 3) The position of the epicoracoid and coracoid of monotremes is similar to that of 'epicoracoid' cartilage and coracoid in lizards and *Sphenodon*. These arguments may now be examined.

1. The epicoracoid: In monotremes this is an independent ossified element. The so-called epicoracoid of lizards and *Sphenodon* is a cartilage which, as the development of the shoulder-girdle of the lizard would tend to show, is merely a peripheral portion of the primary girdle into which ossification has not extended. It is found not only anterior, but ventral to the 'coracoid.' Cartilage is assumed to have occupied both these positions in many of the extinct primitive and mammal-like reptiles. This cartilage seems to have been somewhat variable in its development; some well-preserved Permian girdles show little possibility of cartilage anteriorly. As Hanson admits, there is no evidence that a bone was ever present in this position.

This cartilage Gregory and Hanson compare with the epicoracoid bone in the monotreme. But the monotreme also preserves an anterior and ventral peripheral cartilage which borders the epicoracoid bone exactly as the 'epicoracoid' cartilage of reptiles borders the anterior coracoid element (Parker, '68, p. 192). The value of any comparison that may be drawn between a variable peripheral reptilian cartilage and the distinct and ossified epicoracoid of the monotreme would seem to be open to serious question, especially in view of the fact that the cartilage itself is present in the latter form.

2. The muscular relations of the elements: Two objections may be raised to a comparison of the muscular origins of the coracoid plate of lizards and *Sphenodon* with those of the coracoid elements of the monotreme. If the single coracoid of modern reptiles replaces functionally the two primitive elements of the coracoid plate, the musculature borne by the single modern reptilian bone would have been distributed between two elements in the primitive forms and in the ancestors of the mammals. Further, if, in the evolution of the mammals, a portion of the musculature of the coracoid plate has moved upward on to the scapula, as it is generally believed to have done, marked divergence in the monotreme from the primitive condition of the musculature of the coracoid elements would have been expected.

Gregory compares the muscular relations of the 'coracoid' of *Sphenodon* with those of the coracoid of the monotremes, and states quite correctly that the coracobrachialis and biceps arise from the supposedly homologous bones in either case. But differences are also found. In the monotreme the epicoracoid shares in the origin of the biceps. Further, the *Sphenodon* 'coracoid' bears a large portion of the origin of the supracoracoideus. This muscle is the homologue of three muscles in the monotreme, the supraspinatus, infraspinatus, and supracoracoideus proper.<sup>1</sup> These derivatives are found upon the epicoracoid and scapula of the latter form. Hence the musculature of the *Sphenodon* 'coracoid' is distributed between all three elements of the primary shoulder-girdle of the monotreme.

The musculature of the 'epicoracoid' cartilage of *Sphenodon* is compared with that of the monotreme epicoracoid by Gregory. As he states, this cartilage in *Sphenodon* bears a portion of the origin of the supracoracoideus; it also gives origin to part of the scapulohumeralis anterior. In the monotreme the supracoracoideus proper originates from the epicoracoid bone, but the supraspinatus and infraspinatus, which are probably also derived from the reptilian supracoracoideus, are associated not with this bone, but with the scapula. The teres minor, the

<sup>1</sup> This matter is discussed more fully in a paper shortly to appear in the Bulletin of the American Museum of Natural History, 1922.

probable homologue of the reptilian scapulohumeralis anterior, does not arise from the epicoracoid, but from the scapula. Thus the musculature attached to the reptilian 'epicoracoid' cartilage is found in the monotreme to be attached for the most part to the scapula.

The muscular relationships, then, can scarcely be said to prove the homologies that have been suggested. It is of interest to note that Watson has used these relationships, by the seemingly more legitimate comparison of the monotreme muscles with their probable distribution in the mammal-like reptiles, in arriving at an entirely different conclusion.

3. The argument from the position: Gregory is followed by Hanson in comparing the spatial relations of the elements of the monotreme with those of the modern reptilian shoulder-girdle. The questionable value of this comparison has been referred to on page 42.

The so-called epicoracoid of lizards and *Sphenodon* is widely excluded from the glenoid, and the same is said by Gregory and Hanson to be true also of the epicoracoid of the monotreme. Although the latter element is excluded from the glenoid, it can scarcely be said to be 'widely' excluded, if viewed from the outer aspect. If this argument of exclusion from the glenoid be applied to the therapsids, it will be found to apply to the anterior coracoid element (Watson). This agrees with Broom's and Watson's homology of this element with the monotreme epicoracoid.

In modern reptiles the glenoid is bordered by the surviving anterior coracoid element. But in the higher therapsids the glenoid cavity is bordered by the posterior coracoid. This is also the case with the true coracoid of monotremes, which again bears out the conclusion of Broom and Watson.

The position of the two bony elements of therapsids is almost identical with that of the two bony elements of the monotremes. The primitive posterior coracoid of the therapsid occupies the same position as that of the true coracoid of the monotreme. The primitive anterior element occupies essentially the same position as that of the monotreme epicoracoid. To make a

comparison between these two related groups under the theory of Gregory and Hanson, one must imagine that, 1) while the anterior 'epicoracoid' cartilage was retained, an ossified epicoracoid made its appearance behind it and took up the position of the anterior coracoid of therapsid. Also, 2) that the anterior coracoid moved into the exact position of the posterior coracoid of therapsids, and, 3) the posterior coracoid of the mammal-like reptiles, which had grown constantly in size throughout the therapsid series, was replaced in all its relations by the anterior element, and disappeared completely.

If, however, one concludes with Broom and Watson that the mammalian coracoid is the primitive posterior element, the practical identity of position between the two elements of monotremes and the two bony elements of the mammal-like reptiles is quite evident.

Hanson says: "It may be pertinent to ask, if the coracoid process of placental mammals is the posterior element of Permians, how did it get to the anterior side of the glenoid? It is hard to imagine any rotation or migration of this element which would bring it from a position distinctly posterior of the glenoid to its present distinctly anterior position." It may be noted in this regard that the Permian posterior coracoid is never 'distinctly posterior of the glenoid.' It carries half the glenoid surface, while the therapsid posterior coracoid occupies completely, or almost completely, the ventral half of the glenoid. The mammalian coracoid is not always 'distinctly anterior' to the glenoid. In the monotreme it occupies the ventral half of the glenoid, as does the therapsid posterior coracoid. The two elements seem clearly homologous; we can trace nearly every gradation between the placental coracoid process and the posterior coracoid of the primitive Permian reptiles. Further, since the modern reptilian 'coracoid' is the anterior element, it is not the homologue of the true mammalian coracoid, but of the epicoracoid of monotremes.

## SUMMARY

1. The monotreme shoulder-girdle should be compared with that of the forms from which the mammals are believed to have been derived (therapsids), and not with that of unrelated modern reptiles.

2. The mammalian coracoid is the posterior coracoid element of primitive and mammal-like reptiles, as stated by Broom and Watson.

3. The modern reptilian 'coracoid' is homologous with the primitive anterior element and the epicoracoid of monotremes.

4. The so-called epicoracoid of modern reptiles is a cartilage which may be compared with a variable anterior and ventral cartilage in extinct reptiles and a similarly placed cartilage in monotremes.

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Resumen por el autor, Walter E. Sullivan.

La función de los discos articulares.

Los discos articulares se encuentran bajo una forma simple y diagramática en el cráneo del bacalao, *Gadus morrhua*. Relacionando estos discos con los hallados en el hombre, el autor ha encontrado que los del último pueden reducirse á simples formas geométricas, las cuales indican que en todos los casos compensan las desigualdades de las superficies opuestas con el fin de mantener la estabilidad, careciendo de otra función.

Translation by José F. Nonidez  
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## THE FUNCTION OF ARTICULAR DISCS

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TWO FIGURES

The present work is incidental to a study of the skull of the cod, *Gadus morrhua*, by dissection rather than by maceration. While making this dissection several examples of complete articular discs were found, and these seemed to clarify to some extent the views held in regard to the function of the discs.

Several general views are held in regard to the function of the discs. These are well reviewed by Fick and need be referred to only briefly here. The one most generally held is that they compensate for incongruities of the opposed surfaces. It has been held that they represent vestigial skeletal elements; that they make the lubrication of the joints more simple; that they are shock absorbers, and that they increase the range of movement of the joint. The elementary fact that in the structure of joints only two factors, mobility and stability, may be considered is frequently lost sight of.

It may be pointed out that relatively little work has been done on the joints in fishes. Petersen's work is a marked exception. In some ways the skull of the cod is more complex than the skull of the vertebrates grouped above it and diarthrodial joints exist between skeletal elements which in higher forms are united through a synarthrosis. The maxilla and premaxilla may be cited as examples.

The discs in man are frequently irregular, vary in a slight degree in different individuals, and in this way have their primary function somewhat obscured. In the cod, on the contrary, they are relatively simple and diagrammatic in outline, and but little doubt is left in regard to their function. Here an attempt will

be made to correlate the discs found in the cod with those found in man, by reducing them to simple geometrical figures.

Two general types of discs are found in the cod, both of which are biconcave and elliptical in the outline of the margins. In one type the long axes of the concavities are parallel to each other; in the other type the long axes of the concavities are at right angles to each other.

In man the discs occur in various shapes. There is the biconcave type of the temporomandibular joint; the irregular but usually concavoconvex disc of the sternoclavicular joint; the cuneiform disc of the wrist-joint. The menisci of the knee-joint are in pairs, so that the result is that of a biconcave or planoconcave disc.

In the formation of joints there may be all types of shapes in the articular surfaces. The greatest degree of mobility and the smallest degree of stability would be attained by opposing two convex surfaces, preferably segments of spheres, to each other. The almost complete absence of stability precludes the use of this arrangement. In the shoulder-joint we have an approach to the opposition of a plane and a convex surface. The shallow glenoid cavity and the weakly developed labrum add but little to the stability of the joint. In the hip-joint, on the contrary, there is a well-balanced condition of mobility and stability.

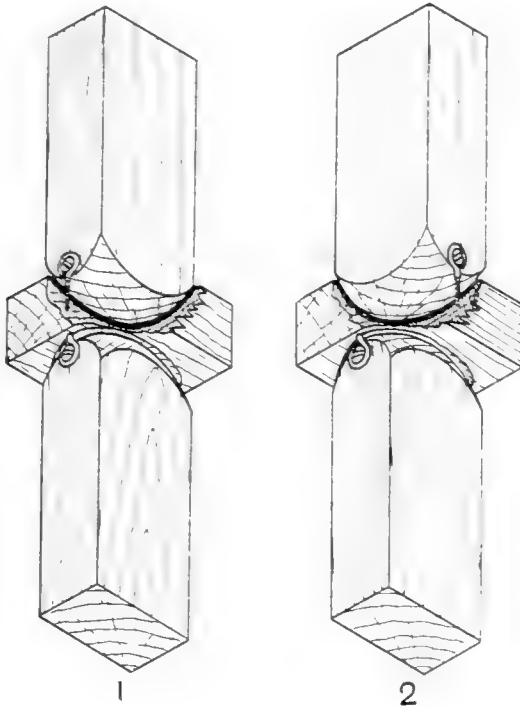
By the addition of an articular disc, stable joints may be established between surfaces that would otherwise lack stability. First, by way of illustration, the condition of a diarthrosis in the midline may be considered. The opposing surfaces of necessity will be symmetrical. If two plane surfaces are opposed there is stability but little mobility. If the opposing surfaces are convex, the reverse is true. If both mobility and stability are desirable, it is practically reduced to a condition of two convex articular surfaces with a biconcave disc interposed.

The presence of a biconcave disc with the long axes of the concavities parallel may be considered. Figure 1 illustrates this type. In man the temporomandibular joint is the nearest approach to it. In general it cannot be held that this type holds any very decided advantage over the condyloid joint. The range



of movement is usually slightly greater without any loss in stability and a small amount of voluntary rotation is usually added to the movements permitted in the condyloid type.

Then there is the type where the disc is biconcave or concavo-convex, but with the long axis of one articular surface at right angles, or nearly so, to the long axis of the other articular surface.



Figs. 1 and 2

This is met with in a simple form in the joint between the maxilla and the premaxilla in the cod and in a modified form in the sternoclavicular joint in man. This gives a very stable type of joint which is essentially biaxial, but which usually permits a small amount of rotation. Figure 2 illustrates this type. Movements in one plane may take place by one of the bones' moving on the disc, and movements in the other plane would then take place by the same bone and the disc moving on the

other bone. If the movements are unrestricted, as is the case in fishes, either bone may move on the disc, or the disc and either of the bones may move on the other bone. In all of these movements the areas of contact are at a maximum and a large degree of stability is assured.

It may be pointed out that the long axes of the disc of the sternoclavicular joint are not usually at right angles to each other; but in general it can be correlated with the principles just stated. On the basis of the movements permitted, it seems that this joint should be grouped with the enarthrodial joints.

The articular disc at the wrist-joint is in many ways the most interesting of the accessory joint structures. Its shape is changed but little during the ordinary range of pronation and supination, and so the shapes of the opposed surfaces at the wrist-joint remain about constant and its stability is not impaired during the excursion of the radius upon the ulna.

#### SUMMARY

It seems hardly necessary to discuss the discs as shock absorbers. The presence of joints in general may be considered as a factor in the absorption of shock, but the articular discs are not placed in positions that would indicate that they play any important part. Indirect evidence is offered by the fact that among the bones most frequently fractured are those associated with articular discs.

Lubrication is a function of the synovial membrane. Opinion seems about equally divided as to the presence of synovial membrane over the articular discs. My personal conviction is that it is present, for at the wrist-joint I have succeeded in isolating it as a gross structure by dissection. Increased lubrication, however, is effected most simply by folds of the synovial membrane. The discs present unbroken surfaces.

It is hardly proper to refer to them as vestigial skeletal elements, for they are themselves skeletal elements and are found in vertebrates as low as the fishes, and here, indeed, in their greatest perfection.

Freedom of movement depends on the shape of the articular surfaces and upon the length of the capsule and accessory ligaments. In some cases the adjacent muscles may play a part. The discs in themselves do not increase the range of movement, but only contribute to the stability of the joints during increased movement dependent on other factors.

The articular discs may be reduced to geometrical forms which compensate for the incongruities of the opposed surfaces to the end of maintaining stability. They should be considered stabilizers only. The other functions attributed to them either do not exist or are incidental to their primary function. My own conclusion is that the other functions do not exist.

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The two drawings for figures 1 and 2 of this paper were made by W. A. Werrel, to whom I wish to express my thanks.

Resumen por los autores, P. N. Johnstone, F. H. Wakefield y  
H. M. Currey.

Sobre la vascularidad relativa del músculo cardíaco y de las  
fibras de Purkinje.

En la presente investigación se ha comparado la vascularidad de las fibras de Purkinje y del músculo cardíaco del corazón de la ternera. Los autores han encontrado que las fibras de Purkinje están mucho menos vascularizadas que el músculo cardíaco adyacente. El haz principal es un poco más vascular que los miembros. En el haz de His los vasos precapilares están confinados, en general, al tejido situado entre las fibras de Purkinje mientras que los capilares están dispuestos en su periferia. En el haz principal y en las porciones superiores de los miembros los vasos sanguíneos no penetran dentro de las fibras de Purkinje. En las porciones inferiores de los miembros y en las bandas moderadoras las fibras están atravesadas por vasos sanguíneos. A causa de la diferencia de la vascularidad entre el músculo cardíaco y las fibras de Purkinje, los autores creen que estas no representan probablemente un tejido especializado para el mismo tipo de contractilidad del músculo cardíaco.

Translation by José F. Nonidez  
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## ON THE COMPARATIVE VASCULARITY OF HEART MUSCLE AND OF THE PURKINJE FIBERS

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TWO FIGURES

Curran, in describing a constant bursa in relation to the Bundle of His, states: "The existence of the bursa tends to prove that either the auriculo-ventricular bundle does not contract at all, or that it contracts in a different way and at a different time from the contraction of the ventricles." Erlanger, in studying the physiology of Purkinje tissue, observed, after numerous trials, a case in which there seemed to be independent movement of false tendons. His work was done on the calf's heart. Regarding the contractility, he says: "Owing to the difficulty of reaching the false tendons and to the fact that they move with the part of the heart to which they remain attached, it was found impossible to record satisfactorily their movements. Throughout the research, however, the false tendons have been closely observed for the purpose of determining whether they contract. The cut tendon lying against the smooth, moist endocardium, or hanging freely into the cavity of the ventricle would certainly have moved perceptibly had any of its elements contracted. In only one case did the false tendons seem to move independently of the heart wall. In this case the false tendons contained no heart muscle."

If the Purkinje fibers represent tissue specialized for the same type of contractility as heart muscle, one would expect them to have a vascular supply equal in abundance to that of heart muscle. We have, in this study, compared the vascularity of the Purkinje fibers and of heart muscle.

The accompanying illustrations are from drawings made with the camera lucida. The sections were made from calves' hearts, the coronary arteries of which had been injected with India ink. The hearts were injected at the slaughter-house immediately after the animals had been killed, and in all cases were soft and pliable; that is, they were not yet in a state of contracture. In most cases the auricles were fibrillating. The injections were all done with a syringe, only a medium pressure being used. If the heart was injected before it was in a state of contracture, it was not found necessary to use high pressure in order to obtain a capillary injection.

The first step in the injection was to split the right posterior portion of the aorta longitudinally. Care was taken to split only the aortic wall. By spreading the cut surfaces of the aorta, the sinuses of Valsalva were exposed. The left posterior sinus was then circled with a sharp knife from the inside of the aortic wall. The sinus was then freed from the underlying connective tissue, and the cannula was inserted, the tie being placed around the aortic border of the sinus. This procedure was duplicated on the anterior sinus. In this manner every branch of either coronary artery was injected, and at the same time the ink was, for the most part, kept out of the left ventricle. The heart was then immediately opened, the larger blood vessels being clamped with hemostats, and the His bundle was dissected out. Seepage of ink from the smaller blood vessels was controlled by gentle sponging. Some cardiac muscle adjacent to the His bundle was left attached to it. Carnoy fixation was used, and the tissue was imbedded in paraffin. Sections were cut parallel to the long axis of the Purkinje fibers and at a thickness of  $25\mu$ . These sections were then stained with hematoxylin and eosin.

Figure 1 is a low-power drawing of a section made from the left limb of the bundle of His. Comparison of the vascular supply can be made with that of the cardiac muscle which has been left attached. It can be easily made out in this section that the Purkinje fibers are much more scantily supplied with blood vessels than is the adjacent heart muscle. In fact, one of the most striking differences is the degree of vascularity. It can also

be made out that the precapillary vessels are confined, in the main, to the tissue between the Purkinje fibers, the capillaries being arranged around their periphery.

Figures 2A and 2B are high-power drawings of small areas of the Purkinje fibers (2A) and heart muscle (2B) shown in figure 1. By comparing them, one can see how scanty is the vascular supply of the Purkinje fibers in contrast to the abundant supply of the heart muscle.

The question at once arises: Is the comparative vascularity between heart muscle and the Purkinje fibers, as shown by the above illustrations, representative of the structure as a whole? A study of the right limb of the bundle reveals substantially the same degree of vascularity as is found in the left limb. Sections from the main bundle show essentially the same vascular arrangement as in the limbs, with a slight vascular increase.

Lydia DeWitt, arguing that Purkinje fibers are syncytial in character, states that no blood vessels or nerves seem to penetrate them. In not a single instance do our specimens show blood vessels penetrating the Purkinje fibers in the main bundle. In the lower portions of the limbs and in the moderator bands, where the fibers increase in size, our specimens show them pierced by blood vessels.

Meigs, in a study of the capillaries of the human heart, says: "The presence of the large capillaries and the penetration of the muscular fibers by the capillaries indicate a provision for the blood supply of the heart even more bountiful than that of the other organs." He studied both injected and uninjected specimens and showed illustrations proving this contention.

As the vascular supply of the Purkinje fibers is very scanty in comparison to heart muscle, it is probable that they do not represent tissue specialized for the same type of contractility as heart muscle.

We wish to express our thanks to Dr. E. R. Clark for his assistance during the progress of this work. We wish further to acknowledge the aid of Mr. G. T. Kline, who made the final drawings for the illustrations.

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# PLATE 1

## EXPLANATION OF FIGURES

All drawings were made with the camera lucida set at table level.

1 Drawing of a section from the left limb of the bundle of His of the injected calf's heart. Some cardiac muscle adjacent to the bundle of His has been left attached. The sections were cut parallel to the long axis of the Purkinje fibers and at a thickness of 25  $\mu$ . They were stained with hematoxylin and eosin.  $\times 72$ .

2 Figures 2A and 2B are high-power drawings of small areas of the Purkinje fibers (2A) and heart muscle (2B) shown in figure 1.  $\times 300$ .



# VASCULARITY OF HEART MUSCLE

P. N. JOHNSTONE, F. H. WAKEFIELD, H. M. CURREY

PLATE I



2



Resumen por W. H. Lewis, por el autor C. S. Beck.

La distribución relativa de los clasmotocitos en los diversos órganos del embrión de pollo de siete días.

En todas aquellas estructuras del embrión de pollo de siete días que contienen tejido reticular laxo existen numerosos clasmotocitos. Su número parece variar con la cantidad de este mesenquima laxo. Abundan especialmente inmediatamente debajo de la epidermis, del revestimiento endodérmico del canal alimenticio y del revestimiento mesotelial de las cavidades pleural y peritoneal. Existen muchos entre las fibras esqueléticas musculares y las fibras lisas, siendo raros en el músculo cardíaco. Abundan también en el mesenquima laxo de los pulmones y la córnea, alantoides y amnios; son raros en el mesonefros, metanefros y el bazo, faltando en la esclerótica, hueso, cartilago, retina y lóbulos ópticos. El autor no encontró ninguno en el hígado.

En algunas partes del cerebro, aun cuando en este órgano se supone que no existe mesenquima laxo, son abundantes los clasmotocitos, como ocurre en la corteza cerebral y en el tálamo. Son también numerosos en los plexos coroideos. Su distribución fué determinada mediante el examen de frotos vivos teñidos con rojo neutro. Los granos y vacuolas se tiñen rápidamente con el rojo neutro, comunicando al clasmotocito un color rojo patente, que contrasta con el de los otros tipos de células. Esta coloración de las vacuolas grandes tan abundantes en el clasmotocito permite diferenciarlos fácilmente de todos los demás tipos en los frotos de células vivas.

## THE RELATIVE DISTRIBUTION OF CLASMATOCYTES IN THE VARIOUS ORGANS OF THE SEVEN- DAY-CHICK EMBRYO

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The type of cell known as the clasmatocyte is classified under the general group of phagocytic cells to which Metchnikoff<sup>1</sup> gave the term *macrophage*. These cells have received various designations: for example, *clasmatocytes* by Ranvier,<sup>2</sup> *ruhende Wanderzellen* (Clasmatocyten) by Maximow,<sup>3</sup> *cellules rhagiocrines* by Renaut,<sup>4</sup> and *macrophages* by Evans and Scott.<sup>5</sup> To this group also belong the macrophages which inhabit the serous cavities of the body.

The method employed in determining the relative distribution of clasmatocytes consisted of an examination of spreads of living tissue from seven-day-chick embryos which had been exposed to a solution of neutral red. Pieces of tissue, approximately a millimeter or less in each dimension, were dissected from various organs under the binocular microscope and freed entirely from the surrounding tissues. The pieces were then immersed for several minutes in a mixture of neutral red and Locke's solution in a concentration of 1:20,000, mounted, and examined immediately as living spreads.

<sup>1</sup> Metchnikoff, Elias, 1892. *Leçons sur la pathologie comp. de l'inflammation*. Paris.

<sup>2</sup> Ranvier, L. 1899. Des clasmatocytes. *Archiv. d'Anat., Micr.*, T. 3, pp. 123-139.

<sup>3</sup> Maximow, A., 1906. Ueber die Zellformen des lockeren Bindegewebes. *Archiv. f. micr. Anat.*, Bd. 67, S. 680-757.

<sup>4</sup> Renaut, J., 1907. Les cellules connectives rhagiocrines. *Archiv. d'Anat., Micr.*, T. 9, pp. 495-606.

<sup>5</sup> Evans, H. McL., and K. J. Scott, 1921. On the differential reaction to vital dyes exhibited by the two great groups of connective-tissue cells. *Contributions to Embryology*, Carnegie Inst. Wash., Pub. 273.

The clasmatocyte of the chick, as seen in tissue treated by this method, is a relatively large cell, varying from about 10 to 20  $\mu$  in diameter. Numerous processes or pseudopodia extend from the cell-body, and these vary in number and size according to the structures surrounding the cell. In a fluid medium the clasmatocyte may be perfectly ovoid in shape; when lying between muscle-fibers, it usually possesses two processes which extend between the fibers. When the clasmatocyte is relieved of the stress exerted by the surrounding structures, as when it flows from its position between two muscle-fibers, its processes are retracted and it becomes ovoid in shape. In other places the processes of the cell are variable in number and extend irregularly from the cell-body. The cytoplasm is always relatively abundant and contains vacuoles which stain with neutral red. These vacuoles form one of the most conspicuous morphological features of the clasmatocyte. When treated with neutral red they become markedly colored; when examined unstained under the microscope they stand out prominently and somewhat resemble globules of fat. In tissue that has been run through alcohol in the process of staining, the globules are dissolved, as a result of which it is almost impossible to recognize the clasmatocyte in ordinary sections.

The clasmatocyte is, as a rule, mononuclear. The nucleus is eccentric in position and in the living cell remains unstained. It is not very uncommon, however, especially in the submucosa of the proventriculus where it was particularly observed, to find a clasmatocyte containing two nuclei. In this location the cell shows the greatest variation in morphology; the cytoplasm is usually packed with vacuoles, there may be one or two nuclei, and the processes of the cell assume irregular contours. Here, also, the cells have been observed in process of division. In most clasmatocytes that are flattened out in tissue cultures, a centrosphere, or an area free from vacuoles and fat globules, can be seen to occupy the center of the cell. The centrosphere is comparatively large and finely granular and in its center, in fixed material, is seen often a single or double centriole. The periphery is continued into the surrounding cytoplasm and

with certain fixations distinct radiations can be seen extending from the centrosphere into the cytoplasmic framework. The nucleus is always crowded off to one side of the cell and may be separated from the centrosphere by vacuoles.

In all those structures of the embryo in which mesenchyme exists, especially if it exist in a fairly loose or reticular form, clasmatocytes are abundant. This loose or reticular mesenchyme is present in the relatively vast areas lying directly beneath the epidermis, beneath the entoderm of the entire alimentary tract, and beneath the mesothelial lining of the pleural and peritoneal cavities. A bit of tissue taken from any of these areas, it matters not whether from the subcutaneous area, the submucosa, the subserosa, the parietal wall, or the supporting mesentery, contains clasmatocytes in the greatest abundance. This condition is common in the spreads that have been mounted with considerable fluid and simply shows the ease with which the clasmatocyte can be removed from its position. As the differentiation of mesenchyme proceeds to the development of specialized structures, the number of clasmatocytes progressively decreases. This is shown strikingly in the successive stages of bone formation, the reticular areas of mesenchyme containing numerous clasmatocytes, while the contiguous and more highly differentiated areas of cartilage and bone are entirely devoid of them. This condition is shown also in the sclerotic coat of the eye, where the mesenchyme destined to form this structure has become, at the age of seven days, semicartilaginous in character and is entirely free from clasmatocytes.

The relationship between the degree of specialization of mesenchyme and the number of contained clasmatocytes is also shown strikingly in the musculature of the seven-day-chick embryo. At this age the musculature markedly resembles the soft, semifluid mesenchyme from which it has arisen, and one might expect to find numerous clasmatocytes in such structures. Both the skeletal and the non-striated varieties of muscle-fibers are surrounded by abundant mesenchyme in which clasmatocytes are very numerous. In the case of the heart, this specialization of structure has advanced to a much higher degree than that

of the other two varieties of muscle. The anastomosing muscular trabeculae make up by far the greater mass of the heart substance and the mesenchyme between the trabeculae has become specialized connective tissue of a functional character. To demonstrate the presence of clasmatocytes in the heart, either in the epicardium, endocardium, or myocardium, may require careful examination of numerous living spreads, but an occasional clasmatocyte can be found, usually more readily in the epicardium and the endocardium than in the myocardium, where they are quite rare. The same condition that exists in the heart is found also in the large vessels connected with it.

In the mesodermal layer of both the inner and the outer walls of the allantois, clasmatocytes are abundant, in numbers approximating those found in the loose or reticular mesenchyme occurring in the subcutaneous tissue and in the submucosa of the gut. The same is true of the amnion, where smooth muscle exists in a relatively loose or reticular form. Examination of the yolk sac does not reveal their presence. This is rather to be expected, in view of its histological structure; the yolk sac is essentially an entodermal structure and the picture under the microscope is greatly blurred by the presence of large glandular cells containing much fat.

The lung, embryologically, is an outgrowth from the post-branchial pharynx and at this age (seven days' incubation) consists essentially of three or four entobronchi embedded in an extensive, loose mesenchymal matrix. Clasmatocytes are almost as numerous in this loose mesenchyme of the lung as in the mesenchyme of the subcutaneous area.

The presence or absence of clasmatocytes in the structures of the eye corresponds again to the presence or the absence of loose reticular mesenchyme. At this age the mesenchyme of the sclerotic coat has become highly differentiated and contains no clasmatocytes. The choroid coat is supposed to be a differentiation of the mesenchyme surrounding the optic cup. Under the microscope it appears to be made up only of highly differentiated pigmented cells. The loose reticular mesenchyme has disappeared from this structure and clasmatocytes are correspondingly

absent. The cornea develops from two sources: the external epithelium is derived from ectoderm, the cornea propria from the surrounding mesenchyme. This mesenchyme begins to penetrate the substance of the cornea propria at the seventh day and forms the so-called corpuscles of the cornea. Clasmatoocytes are abundant in the cornea and can be found upon examination of both the inner and the outer surfaces of the structure.

The mesonephros is the principal organ of excretion during the embryonic life of the chick, and at the end of seven days of incubation it forms the greater mass of kidney substance. In conjunction with the well-developed mesonephros and Wolffian duct, there has already developed nephrogenous tissue of the metanephros with its diminutive ureter. As the development of these structures takes place in a mass of mesenchyme, furnishing, as it does, both the capsule and the connective-tissue elements of the kidney, it is not surprising to find an occasional clasmatoocyte in the connective-tissue elements lying between glomeruli or renal tubules. The number of clasmatoocytes present in mesonephros or metanephros is small. They are relatively more abundant in the walls of the Wolffian duct and the ureter than in the kidney substance.

In the spleen great numbers of cells take up neutral red. These cells do not possess the characteristic pseudopodia and the vacuoles of the clasmatoocyte. They are more regular in outline and seem to be of a more fixed nature. They are probably reticular cells. The clasmatoocyte undoubtedly can be demonstrated in the spleen, but careful search is required, as in this organ it does not constitute a conspicuous type of cell.

Clasmatoocytes were not found in the liver. This organ is remarkable in possessing no mesenchyme in the embryonic stages after the fourth day (Minot<sup>6</sup>). With the absence of mesenchyme it is not surprising to find an absence of clasmatoocytes in this organ. The endothelial type of macrophage is represented here by the Kupffer cells, which can be demonstrated in spreads treated with neutral red.

<sup>6</sup> Minot, C. S., 1900. On a hitherto unrecognized form of blood circulation without capillaries in the organs of vertebrata. *Proc. Boston Soc. Nat. Hist.*, vol. 29.

The presence or absence of clasmatoocytes in every structure so far mentioned may be accounted for adequately by the presence or absence of loose reticular mesenchyme existing in that organ. Examination of the nervous tissue shows that the brain and the spinal cord do not parallel this general principle, because clasmatoocytes are not uniformly distributed throughout the nerve substance; they are, in fact, absent from the optic lobes of the brain and from the retina. The cortical hemispheres contain clasmatoocytes and throughout the corpus striatum they are relatively abundant. In the regions of the choroid plexus, in the telencephalon medium, and in the lower portions of the thalamus, clasmatoocytes are quite numerous.

Mention should be made of the existence of a type of cell to which no reference could be found in the literature. These cells vary considerably in size, being approximately  $2\frac{1}{2}$  to  $4\mu$  in diameter and irregularly spherical or ovoid in shape. The nucleus is not a conspicuous structure. The cytoplasm contains granules of variable size which take up neutral red and the cytoplasm itself becomes more or less diffusely colored. These cells are of widespread distribution, occurring in greatest numbers in the brain, retina, and kidney, but their presence can be demonstrated in almost every tissue of the chick.

#### SUMMARY

1. The clasmatoocyte is present in great numbers in all those structures of the seven-day-chick embryo that contain loose reticular mesenchyme.
2. The number of clasmatoocytes present in mesenchymal structures seems to vary with the amount of loose reticular mesenchyme.
3. The brain, excluding the optic lobes and retina, is the only structure supposedly containing no loose reticular mesenchyme in which clasmatoocytes are present.





Resumen por el autor, F. A. McJunkin.

Teñido de la peroxidasa con la benzidina en cortes de parafina de tejidos humanos

Sexta comunicación sobre los estudios de los leucocitos mononucleares de la sangre.

Las secciones en parafina pueden utilizarse para el teñido de la peroxidasa cuando se observa la siguiente técnica en su preparación: Inclusion—1) Piezas de tejido de un milímetro de espesor, fijadas en formol se transportan á una solución de acetona al 70 por ciento en la que permanecerán durante una hora; 2) Acetona, 30 minutos; 3) Benzol, 20 minutos; 4) Parafina, 20 minutos. Teñido—1) Los cortes de 3.5 á 5 micras de espesor se pegan al portaobjetos con albúmina glicerizada dejándolos secar durante la noche á la temperatura de la habitación; 2) Se disuelve la parafina mediante el benzol (20 segundos) y la acetona (10 segundos); 3) Se sumergen los portaobjetos en agua durante unos segundos, se quita el exceso de agua y se tiñen durante 5 minutos en una solución de benzidina (25 centímetros cúbicos de alcohol metílico al 80 por ciento, 100 miligramos de benzidina y, 2 gotas de peróxido de hidrógeno), diluída en el momento de usarla en una ó dos partes de agua destilada; 4) Se lavan los portaobjetos en agua durante 5 minutos y se tiñe con hematoxilina de Harris durante 2 minutos; 5) Nuevo lavado en agua durante 1 minuto y se tiñe con eosina (0.7 por ciento) durante 20 segundos; 6) Se deshidrata con alcohol de 95° (30 segundos) y alcohol absoluto (5 segundos); 7) Xilol; 8) Montaje en bálsamo.

Las células que dan la reacción (color pardo) son las neutrofílicas y los leucocitos y mielocitos eosinofílicos, las células mononucleares de la pulpa esplénica y los sinusoides del hígado, y los leucocitos mononucleares de los vasos sanguíneos.

## PEROXYDASE STAINING WITH BENZIDIN IN PARAFFIN SECTIONS OF HUMAN TISSUE

### SIXTH REPORT OF STUDIES ON THE MONONUCLEAR LEUKOCYTES OF THE BLOOD

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FOUR FIGURES

A practical method for the peroxydase staining of histologic sections originated with Schultze,<sup>1</sup> although it had previously been observed that certain tissues and tissue products developed color compounds in dilute solutions of naphthols and phenols. By the oxydase reaction of Schultze, frozen sections of formalin-fixed tissue are treated with an aqueous solution of alphanaphthol followed by an aqueous solution of dimethyl-p-phenylene-diamine, with the production of a blue color in myeloblastic cells and no color change in cells of the lymphocyte series. Since these two chemicals in the test-tube unite in the presence of an oxydizing substance to form indophenol blue, the supposition is that the reacting granules in the leukocytes perform the function of an oxydizing agent. The outcome of this conception has been that the reactions in the numerous subsequent methods are spoken of as peroxydase or oxydase, according to whether an additional oxydizing substance such as hydrogen peroxide is employed or not.

Guaiac, alpha-naphthol, benzidin, resorcin, hydroquinon, and pyrogallol are some of the more common compounds known to yield color reactions with tissue. Benzidin unites with numerous amines and phenols to form dyes of the azo group. The exact way in which the cytoplasm of certain cells acts on benzidin to form the brown color elicited is not known and the

use of the terms peroxydase and oxydase may ultimately prove inappropriate. These terms have, however, come into general use and the reacting substance in its behavior to elevated temperatures, solubility in alcohol, and in other ways resembles known enzymes. Of the substances used for the reaction, benzidin yields the most precise and intense staining and the colored compound developed permits the application of the common contrast stains.

That the myeloblastic cells with eosinophilic and neutrophilic granules contain an oxydizing enzyme, while the cells of the lymphocyte series do not, has quite generally been accepted. Few statements in regard to the behavior to benzidin, alpha-naphthol and other substances of non-lymphocytic mononuclear leukocytes and of endothelial cells of lymph and blood vessels have been made. Graham<sup>2</sup> observed areas of reacting cytoplasm in endothelial cells lining capillaries especially in the liver, and suggested that such reactions are due to particles of disintegrated myeloblastic cells ingested by the lining endothelium. The author,<sup>3,4</sup> working with peripheral blood, found that many of the mononuclear leukocytes which ingest carbon react to benzidin. The intravascular ingestion of carbon by these leukocytes as well as the phagocytosis of carbon particles in vitro by them is accomplished by adding sodium citrate to a concentration sufficient to inhibit the phagocytic activity of the polymorphonuclear neutrophiles. The benzidin reacting cells of normal peripheral blood are the neutrophiles, eosinophiles and many of those mononuclear cells which are commonly spoken of as 'large mononuclear and transitional' leukocytes.

To determine the reaction of various tissues to benzidin for comparison with cells in blood smears giving the peroxydase reaction, frozen sections of various organs were treated with benzidin but with unsatisfactory results. In the methods recommended only frozen sections or teased bits of formalin-fixed tissue have been available, since the reactive property is destroyed by the usual paraffin or celloidin method of imbedding. In 1918 the author<sup>5</sup> published a method for imbedding tissue in soap in which the granules are preserved. Although the soap

sections have proved superior in some respects to frozen ones, they are inferior to those obtained by paraffin imbedding. The purpose of this paper is to give a method for the preparation of paraffin sections with preservation of peroxydase-reacting granules and to describe the results obtained by the method on human tissue.

### *Method*

The method which preserves the reacting substance in certain mononuclear and marrow types of cells is as follows:

*Imbedding.* 1. Bits of formalin-fixed tissue 1 mm. thick are placed in 70 per cent acetone for one hour.

2. Acetone, 30 minutes.

3. Benzol, 20 minutes.

4. Paraffin, 20 minutes.

*Staining.* 1. Sections 3.5 to 5  $\mu$  in thickness, cut in the usual way, are attached to slides with albumin-fixative and allowed to dry overnight at room temperature.

2. Remove paraffin with benzol (20 seconds) and acetone (10 seconds).

3. Plunge the slide into water for a few seconds, remove the excess of water and apply for 5 minutes a benzidin solution (25 cc. of 80 per cent pure methyl alcohol, 100 mgm. benzidin and 2 drops hydrogen peroxide) diluted at the time of placing it on the preparation with one or two parts of distilled water. Two parts give less intense staining. The alcoholic benzidin solution is permanent.

4. Plunge into water for 5 minutes and stain with hematoxylin (Harris) for 2 minutes.

5. Plunge into water for 1 minute and stain with eosin (0.1 per cent) for 20 seconds.

6. Dehydrate with 95 per cent alcohol (30 seconds) and absolute alcohol (5 seconds).

7. Xylol.

8. Mount in balsam.

In order to determine that none of the peroxydase reacting property of the cytoplasm is destroyed in the embedding process,

a second method is available in which an initial treatment with benzidin is performed. It has no advantages over the one given above and has a tendency to color cells diffusely. The method is as follows:

### *Method*

1. Fresh tissue is fixed in 10 per cent formalin, cut into very thin bits less than 1 mm. thick, and washed in running water for 30 minutes.

2. Two hours in a benzidin solution prepared immediately before placing the tissue in it by mixing 7 parts of acetone with 2 parts of distilled water and 1 part of hydrogen peroxide and adding 2 mgm. of benzidin to each cc. of mixture.

3. Acetone, 30 minutes.

4. Benzol, 20 minutes.

5. Paraffin, 20 minutes.

Paraffin sections are cut in the usual way and stained with hematoxylin and eosin as in the first method.

The reacting cytoplasm which is arranged in the form of more or less definite granules is colored yellow to brown. The initial color formed is blue, but this soon changes to brown. In sections the cytoplasm overlying the nucleus tends to obscure the nuclear staining, but the chromatin of the nucleus appears not to react.

It is sometimes desirable to color by the Berlin-blue reaction hemosiderin which may be present. This is done by placing the benzidin-treated sections for 5 minutes in equal parts 1 per cent potassium ferrocyanide and decinormal hydrochloric acid, washing them in the dilute acid followed by water, and proceeding with the hematoxylin-eosin stain in the usual way. Hemosiderin is colored blue, while any brown color brought out by the peroxydase reaction is unchanged by the test.

In unfixed tissue the reacting substance disappears soon after death, and the rate of its disappearance cannot be measured entirely by the time which elapses. After the tissue has become completely fixed in formalin the intensity of the peroxydase staining slowly decreases.

## TISSUE REACTIONS IN BENZIDIN-PARAFFIN SECTIONS

A control section of all tissues from which conclusions are to be drawn must be made by staining in the usual way a section which has not been treated with benzidin. This is especially important in the case of the liver, spleen, and lymph nodes. The commonest yellowish or brownish pigments are the hemosiderins. An iron reaction may be performed on the benzidin preparation as indicated under the directions for performing the stain.

The action of the benzidin solution is specific in the sense that a few varieties of cells regularly react, while the other cells and tissues show no reaction. Briefly, in human tissue no cells are colored by the benzidin except, *a*) myeloblastic cells with neutrophilic and eosinophilic granules; *b*) certain mononuclear cells which are most abundant in the spleen, and, *c*) mononuclear leukocytes which may be found in the blood vessels of any tissues of the body, but which are seen best in the sinusoids of the liver and the sinuses of the spleen. The reaction of the myeloblastic cells is very intense and requires little discussion.

*Spleen.* A large number of mononuclear cells giving a peroxylase reaction are present. Not only is the number much greater than is found in any other organ, but the benzidin-colored cells are sufficiently numerous to make them a conspicuous feature of the microscopic appearance of the spleen. With low magnification they are seen to occur in the splenic pulp between the lymph nodules, which themselves are quite free from reacting cells. The endothelial cells of the characteristic venules have not been demonstrated to react. It may be stated that the reacting mononuclear cells correspond in location and morphology to those usually termed 'splenic or pulp' cells. They vary in size from slightly larger than neutrophils to two or three times that size. The vesicular nucleus is usually indented or saddle-shaped, but sometimes round. In the distinct cytoplasm occurs the brown granulation and sometimes hemosiderin granules, or ingested carbon particles, red blood corpuscles, lymphocytes, or neutrophils are present. Material ingested by phagocytosis does not alter the peroxylase staining of the cytoplasm about it.

The general cell outline tends to be regular, but some cells present projections in one direction or another. The supporting reticular cells do not react. The endothelium of the larger blood vessels does not color with the benzidin.

It is difficult to determine the relation of the reacting cells to other cells of the blood-containing pulp spaces and especially to cells which may or may not line these spaces. Excluding the reticular cells of the pulp and those lining definite blood sinuses, the reacting cells may constitute a majority of those present in the pulp. Mitoses in the cells colored by the benzidin have been observed. The character of both the nucleus and cytoplasm as well as the tendency to ingest non-bacterial material serves to distinguish these cells from myelocytes. The chief reason that the possibility has not been entertained of the mononuclear leukocytes of the blood and of these reacting cells in the lumina of vessels and in the spleen being of marrow origin is that they lack the regularity in size and shape of myelocytes. Congestion and the collection of neutrophiles in the vessels obscure the normal histologic picture.

In the routine examination of sections of spleen cut through the capsular surface occasionally the mesothelial peritoneal covering is found to consist of cuboidal or columnar cells. These cells do not react to benzidin.

*Liver.* In this organ is found one of the most favorable tissues for the study of cells which line capillary blood vessels. Here it is easier to define accurately the lining endothelial cells than it is in the spleen. Within the sinusoids are neutrophiles and a variable number of free cells of the large mononuclear leukocyte type which react with the benzidin. In a few of the endothelial cells lining the sinusoids and the Kupffer cells there are peroxidase granules diffusely distributed. In a somewhat greater number there are spherical focal areas of reacting cytoplasm located usually near one end of the nucleus. The reacting spherules are not surrounded by a colorless zone or halo and do not have the appearance of particles ingested by phagocytosis.

*Lungs, kidney, pancreas, adrenal, and heart* are other organs which have been examined from a small number of autopsies.



Extravascular neutrophilic and non-lymphocytic mononuclear leukocytes are scanty in these situations when acute and chronic inflammatory lesions are absent. Neutrophiles and an occasional large mononuclear leukocyte with positive benzidin reaction occur within the blood vessels. The cells lining the capillaries of these organs do not react. The spleen, liver, and lymph nodes have been examined from about fifty autopsies.

*Lymph nodes.* Owing to the divergent views of the origin of blood-mononuclear leukocytes and tissue wandering cells, especial interest attaches to the reaction of the cells present in the sinuses and in the germinal centers of lymph nodes. Within the sinuses of a non-inflamed lymph gland there are a few neutrophiles, but usually phagocytic and non-phagocytic large mononuclear cells occur and they are often abundant. The mononuclear ones do not react to benzidin. Numerous glands obtained at autopsy have failed to show a trace of reaction in the large mononuclear cells. The phagocytic cells may contain neutrophiles, lymphocytes, blood corpuscles, carbon particles, or fat droplets, but the cytoplasm about the ingested particles like that of the non-phagocytic ones does not react. The endothelial cells lining the sinuses and the reticular cells extending across them are likewise devoid of reacting granules. The cells of the lymph nodules and the germinal centers do not react. In a few instances a limited number of reacting mononuclear cells have been present in the sinuses. The large mononuclear cells in and near the lymph sinuses which often contain ingested corpuscles, neutrophiles, or lymphocytes are larger and possess a larger amount of eosin-staining cytoplasm than is seen in the peroxydase-staining mononuclear cells of the spleen.

#### DISCUSSION AND SUMMARY

The peroxydase reaction has usually been interpreted as a means for the separation of the reacting myeloblastic cells from the non-reacting cells of the lymphocyte series. In blood smears even a casual examination shows that many of the leukocytes spoken of as 'transitional and large mononuclear' react positively to benzidin. The origin of such mononuclear leukocytes

and of tissue wandering cells has been and is now a matter of controversy that is not likely to be settled by proof of a single kind. Observers agree that these wandering cells and the endothelial cells lining the blood capillaries of some organs and the lymph channels in certain locations are both phagocytic, especially for non-bacterial substances such as fat, disintegrating cells, and blood corpuscles. If the wandering cells are considered derivatives of such attached phagocytic cells, it is at least unnecessary to assume that they acquire a new function when they become free; so far as phagocytic behavior is concerned, the large mononuclear leukocyte group of the blood and these attached cells are alike.

The endothelial cells of organs with an extensive capillary network undergo mitosis in locations where the capillary does not appear to be lengthening and become free in the lumen. From this observation and the close resemblance in morphology especially after the ingestion of carbon the conclusion drawn by the writer<sup>6</sup> was that the large mononuclear and transitional cells of the blood arise in this way. The observations from which this conclusion was drawn were made almost exclusively on the liver where endothelium can be studied to the best advantage. The benzidin preparations show that a few of the endothelial cells of the liver react positively, but the number of peroxydase staining mononuclear cells is so great in the spleen that it seems likely that this is an important source of mononuclear blood cells with benzidin granules.

The absence of peroxydase-staining cells in the lymph glands is considered significant. In blood smears to which a peroxydase stain has been applied there are present cells larger and with more cytoplasm than lymphocytes which have nuclei more vesicular than lymphocytes and which do not react. In normal human blood such non-reacting cells constitute a minority of the leukocytes of the 'large mononuclear-transitional' type. Are such cells derived from the lymph sinuses? Summarizing previous work, McClure<sup>7</sup> has called attention to the conclusiveness of the proof of the local origin during embryonic development of vascular endothelium from mesenchyme. The endothelium

of the lymph vessels may arise in parts of the embryo where there is mesenchyme and with no connection with blood vessels. The blood spaces of the splenic pulp develop in the embryo in close association with the blood vessels. That the cells differentiating in this location in connection with blood vessels are in the adult essentially different from endothelium of the lymphatics is indicated by the response to benzidin.

#### CONCLUSIONS

1. Thin bits of formalin-fixed tissue can be passed through acetone and benzol and embedded in paraffin with preservation in all cells having the property of giving the reaction of the substance on which peroxydase staining is dependent.

2. The cells which react positively to the benzidin are, *a*) myeloblastic cells with neutrophilic and eosinophilic granulations; *b*) mononuclear cells present in the splenic pulp and focal areas in the cytoplasm of some of the endothelial cells of the liver sinusoids, and, *c*) large mononucleated leukocytes within the blood vessels.

3. The mononuclear cells of the splenic pulp which give the reaction are present in the spleen of all individuals, and in morphology they are similar to the peroxydase-staining mononuclear leukocytes within the blood vessels. The irregularity in size and shape of the reacting splenic cells indicates that they are not myelocytes. However, the data obtained by peroxydase staining do not exclude the possibility of some of these cells being myelocytes. The relation of these cells to the vascular endothelium in the location where they occur is not clear.

4. The large mononuclear cells regularly present in the lymph sinuses of lymph glands which become phagocytic and present such a conspicuous feature of certain mild lymph node inflammations do not react to benzidin. The positive identification in other tissues of the cells found in the lymph-node sinuses probably awaits the development of a technic characterizing them.

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## PLATE 1

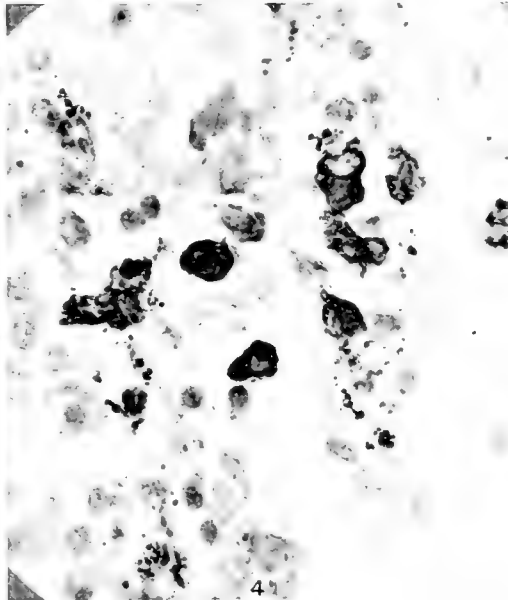
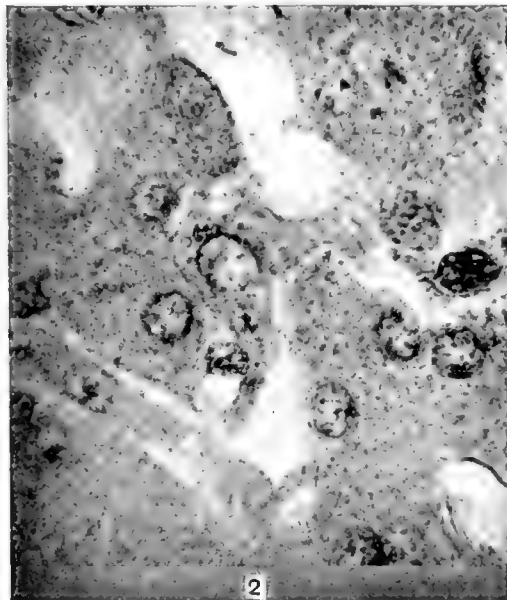
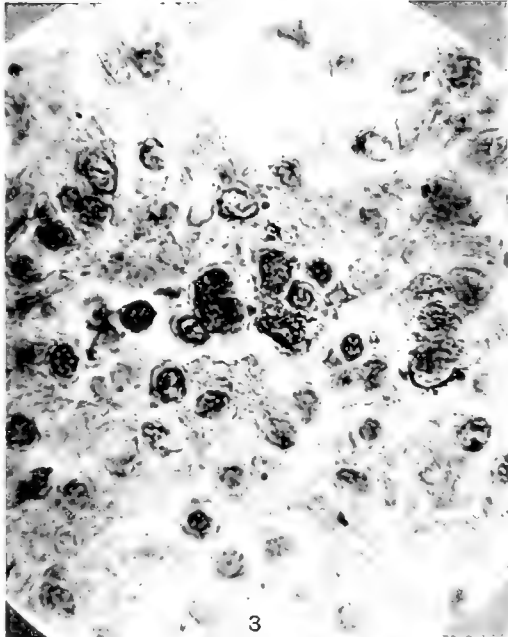
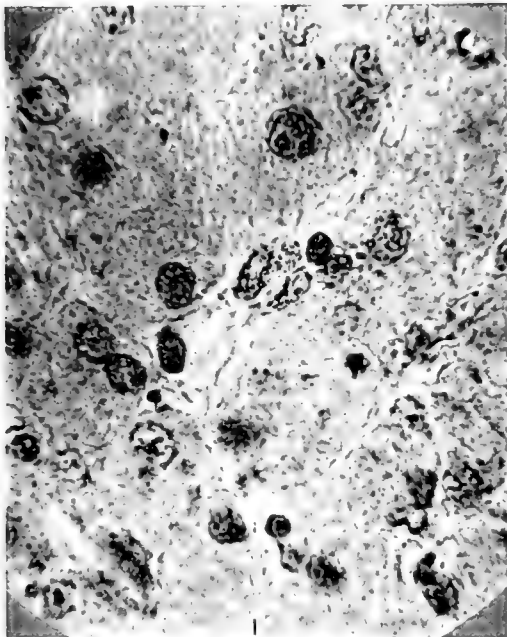
## EXPLANATION OF FIGURES

1 Liver showing an endothelial cell of a sinusoid with cytoplasm containing numerous peroxydase granules. None can be distinguished in the cell on the opposite side of the same sinusoid.

2 Liver with Kupffer cell which contains two circumscribed spherules of reacting cytoplasm.

3 Spleen with three peroxydase-staining mononuclear cells at center of field. Two of them have indented nuclei. Cytoplasm of lymphocytes is homogeneous.

4 Spleen showing mononuclear cells and a neutrophile with peroxydase granules.



Resumen por el autor, A. L. Salazar.

Sobre la forma de la degeneración de los folículos anovulares de Regaud y de otros restos que provienen de los cordones ovígenos del ovario de la coneja.

La aplicación del método tanoférrico al ovario de la coneja ha permitido al autor por vez primera el poner en evidencia el proceso de degeneración de los llamados folículos anovulares de Regaud y las formaciones análogas. Esta degeneración, que el autor llama "atresia hidrópica" consiste en la acumulación en los intersticios de las células del residuo de una substancia que se tiñe en negro oscuro mediante el método mencionado anteriormente. Esta substancia resiste tenazmente á la decoloración con el ferrocianuro de Weigert.

El magma hidrópico crece progresivamente, disociando al residuo, que finalmente se disgrega. Los restos del magma y unas cuantas células, que han escapado á la destrucción, se extienden en el tejido conectivo. El magma hidrópico parece resultar de la alteración atrésica de una delgada capa del líquido que existe normalmente entre las células del residuo. La membrana propia del residuo se hipertrofia á veces, transformándose en un cordón tortuoso, teñido intensamente en negro; este cordón tortuoso á menudo permanece aislado en el tejido conectivo después de la disgregación del residuo. La hipertrofia de la membrana propia es enteramente análoga á la membrana atrésica de Slavjansky. A veces se encuentra asociada con el proceso hidrópico una acción disociativa del tejido conectivo, siempre muy ligera; este hecho es interesante, porque esta acción disociativa, en mucho menor grado, reproduce un proceso característico de la atresia conectiva fetal de Winiwarter.

## SUR LA FORME DE DÉGÉNÉRESCENCE DES FOLLICULES ANOVULAIRES DE REGAUD ET D'AUTRES RELIQUATS PROVENANT DES CORDONS OVIGÈNES DE L'OVAIRE DE LA LAPINE

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DIX FIGURES

La méthode tannoferrique<sup>1</sup> met nettement en relief la forme de dégénérescence des follicules anovulaires de Regaud et d'autres reliquats épithéliaux dérivés des cordons ovigènes. Le processus involutif de ces formations n'est pas encore connu: tout récemment Gérard<sup>2</sup> écrivait: "Que deviennent ces nodules épithéliaux? Nous n'en avons jamais vu dégénérer." Les formations en question dégénèrent par un processus qu'on peut appeler "hydropisie tannophile." Ce processus consiste dans l'accumulation d'une substance liquide ou pâteuse dont le coagulum de fixation prend une coloration intense d'un noir encre de Chine après fixation dans le liquide de Bouin et traitement par le tannin-fer (fig. 1, 2 et 3). Cette substance est très fortement tannophile, car, non seulement elle se colore très intensivement, mais encore résiste avec ténacité à la décoloration par le ferri-cyanure de Weigert. Quand cette différenciation est poussée très loin, jusqu'à décoloration de la plupart des autres formations tannophiles, la substance hydropique résiste encore; sa tannophilie égale ou excède celle des magma atrétiques. Cette substance hydropique s'accumule de plus en plus dans les interstices entre les cellules (fig. 1), atteint des proportions considérables (fig. 2 et 3) et finit par dissocier le reliquat dont les cellules

<sup>1</sup> A. L. Salazar. Méthode de coloration tanno-ferrique. Compt. Rend. Soc. de Biol., T. 83, p. 1655.

<sup>2</sup> Gérard. Contribution à l'étude de l'ovaire des Mammifères. Arch. de Biol., 30, 2<sup>e</sup> part, p. 371.

périssent noyées dans le magma ou deviennent libres. Ces cellules libres se répandent dans le strome conjonctif; parfois un certain nombre de cellules s'entourent d'une membrane propre et reconstituent un nodule plus petit (fig. 5 et 9). Ce dernier fait n'est pas constant. Nous ignorons la destinée de ces cellules. Dans certains cas la membrane propre du reliquat, que la méthode tanno-ferrique dessine nettement en noir, s'hypertrophie à l'un des pôles du reliquat, devient flexueuse et se colore intensivement en noir (fig. 4 et 5); ce fait est entièrement comparable à l'hypertrophie de la membrane de Slavjansky dans la période agénique de l'atrésie (période post-chromatolytique). La forme du cordon, sa tannophilie, etc., sont absolument identiques; il n'y a qu'une différence de dimensions, plus réduites dans les reliquats.

Dans certains cas le processus de dégénérescence est plus complexe. Outre l'hydropisie tannophile, il existe une activité dissociante du tissu conjonctif, qui rappelle l'atrésie conjonctive de Winiwarter, mais cette activité dissociante du tissu conjonctif est ici très discrète et son rôle secondaire (fig. 4 et 7). Ce phénomène est intéressant, car il représente chez l'adulte un processus caractéristique de la vie embryonnaire de l'ovaire. Ce processus, d'un autre côté, n'est qu'une continuation

Toutes les figures ont été copiées de coupes d'ovaires fixés dans le liquide de Bouin et colorées par la méthode tanno-ferrique.

Figs. 1, 2, et 3 Reliquats épithéliaux en dégénérescence hydropique. Fig. 1, magma hydropique encore peu avancé; fig. 3, magma très avancé; fig. 2, un état intermédiaire.

Figs. 4 et 5 Deux reliquats avec membrane propre, hypertrophiée et flexueuse, analogue à la membrane de Slavjansky atrétique.

Fig. 6 Un autre aspect de reliquat en atrésie.

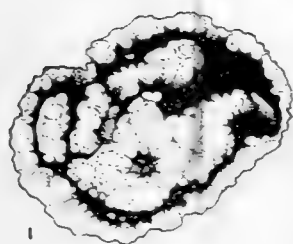
Fig. 7 Reliquat dissocié par l'action du tissu conjonctif.

Fig. 8 Reliquat dont le magma hydropique présente l'aspect d'un cordon flexueux.

Fig. 9 Débris provenant de l'atrésie d'un reliquat avec un petit nodule de cellules intactes.

Fig. 10 Follicule de de Graaf primordial en atrésie avancée: oocyte, débris fibreux de la granulosa, membrane hypertrophiée, sont encore visibles. Cette figure est destinée à éviter la confusion avec l'atrésie hydropique des follicules anovulaires.

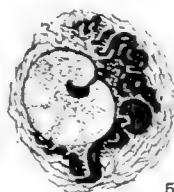




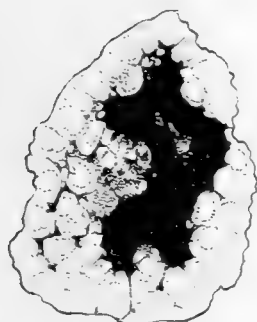
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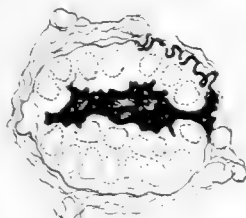
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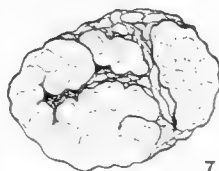
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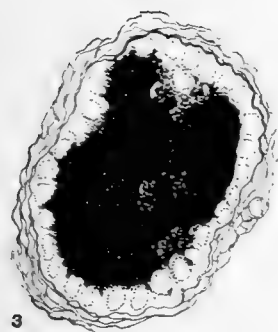
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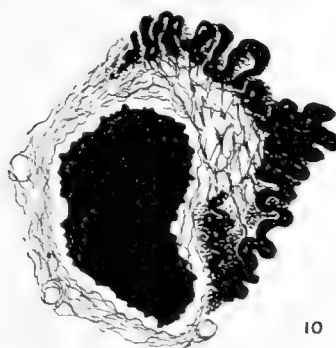
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languissante du processus normal d'amputation des cordons ovigènes.<sup>3</sup>

Le magma hydropique semble dériver de l'accumulation atrétique d'un liquide, qui normalement existe dans les interstices entre les cellules qui constituent le reliquat. Ce liquide, homologue du liquor folliculi interstitiel,<sup>4</sup> se teint en gris ou gris-noir, tandis que le liquor folliculi dans les ovisacs petits et moyens se teint en noir pur.<sup>4</sup>

Les coagula hydropica résultants de la désagrégation des reliquats se répandent dans le strome conjonctif, où ils restent longtemps sous la forme de masses, de blocs, de cordons souvent très longs (fig. 9), parfois capricieux, tout cela coloré en noir intense par la réaction tanno-ferrique. Ces débris tannophiles disparaissent à la fin, canalisés probablement par les lymphatiques ou par les capillaires sanguins, car ceux-ci sont souvent remplis d'une substance très tannophile et abondante.

Les reliquats en pleine dégénérescence hydropique existent dans tous les types d'ovaires: ovigènes, folliculaires, atrétiques, interstitiels-cloisonnés, interstitiels-diffus.<sup>5</sup> Dans les vieux ovaires (type interstitiel) le nombre des reliquats en plein processus hydropique ou encore intacts diminue, tandis que les débris tannophiles résultants des reliquats dégénérés dans les étapes antérieures (ovigène, folliculaire, atrétique) semblent devenir plus nombreux. La destruction des follicules anovulaires semble, donc, plus intense dans les ovaires plus jeunes et se ralentir et s'épuiser au fur et à mesure que l'ovaire vieillit. Nous avons trouvé des reliquats en pleine dégénérescence hydropique logés dans le centre de corps jaunes atrétiques sans vestiges de follicules. Il s'agit probablement d'un reliquat juxtaposé à un follicule, celui-ci ayant disparu plus rapidement. Cependant ces cas suggèrent l'hypothèse de la formation de corps atrétiques autour des follicules anovulaires de certaines dimensions.

<sup>3</sup> A. L. Salazar. Sur les cordons ovigènes, etc. *Compt. Rend. Soc. de Biol.*, 1921, I, p. 235.

<sup>4</sup> A. L. Salazar. Sur le follicule de de Graaf non atrétique. *Compt. Rend. Soc. de Biol.*, T. 83, p. 1658.

<sup>5</sup> A. L. Salazar. Sur l'évolution de l'ovaire adulte de la Lapine. *Compt. Rend. de la Soc. de Biol.*, No. 30, T. 85.

La forme de dégénérescence, qui vient d'être sommairement décrite, rend admissible l'hypothèse d'une action endocrine appartenant au groupe des sécrétions endocrines à forme dégénérative.<sup>5</sup> Parmi les variations que peut présenter le processus, celle qu'on trouve représentée dans la fig. 8 présente un intérêt spécial. Le magma hydropique a pris la forme très nette d'un cordon flexueux plus ou moins long: on dirait qu'un vermicule noir se trouve logé dans le nodule. Il existe du reste des formes de transition qui rattachent cette forme aux magma habituels. Le cordon tannophile en question est analogue à celui qu'on trouve dans les "follicules à cordon tannophile," que nous avons décrits dans l'ovaire de la Lapine.

On ne doit pas confondre les follicules anovulaires atrétiques avec l'atrésie des petits follicules de de Graaf. Dans un état avancé d'atrésie ces follicules présentent dans les coupes colorées par le tannin-fer un aspect qui ressemble d'abord aux follicules anovulaires en état avancé de dégénérescence (fig. 10).

L'absence d'hypertrophie de la thèque ne peut servir pour établir la distinction, car l'atrésie des follicules très petits n'est pas suivie de la formation de corps jaunes atrétiques. D'un autre côté, les follicules anovulaires présentent souvent une hypertrophie de la membrane propre entièrement analogue à celle que subit la membrane de Slavjansky. Cependant la distinction est presque toujours facile; les follicules véritables présentent des débris fibreux reliant le magma atrétique oocytaire à la membrane folliculaire hypertrophiée; le magma noir oocytaire est presque toujours reconnaissable, etc.

<sup>5</sup> A. L. Salazar. Les corpuscules concentriques de la granulosa atrésique de la Lapine. Compt. Rend. Soc. de Biol., T. 84, p. 387.

Resumen por el autor, Charles E. Buell.

### Notas sobre técnica.

#### I. Un método para preparar embriones jóvenes montados enteros.

El embrión se fija en formol (10 por ciento) durante doce horas, lavándole en agua y tiñéndole con hematoxilina. Se destiñe en 5 por ciento ó lo por ciento de NaOH hasta que haya desaparecido todo el color azul y la preparación adquiere una tinta parda clara. Se deshidrata y se monta en bálsamo. El resultado se asemeja al obtenido mediante la impregnación argéntica.

#### II. Disección en parafina.

El embrión inyectado se incluye en parafina del modo ordinario. La disección se lleva á cabo en el bloque de parafina bajo el microscopio binocular, con fuerte aumento. Se disuelve la parafina en xilol, y se monta el embrión en bálsamo.

Translation by José F. Nonidez  
Cornell Medical College, New York

## NOTES ON TECHNIQUE

### I. A METHOD OF PREPARING WHOLE MOUNTS OF EARLY EMBRYOS

#### II. DISSECTION IN PARAFFIN

CHARLES ELBERT BUELL, JR.

*Anatomical Laboratory of the Johns Hopkins University*

### I. A METHOD OF PREPARING WHOLE MOUNTS OF EARLY EMBRYOS

In the preparation of total mounts of young embryos, both injected and uninjected, certain well-known difficulties are encountered. If the embryos are mounted unstained, the tissues become too transparent in the clearing reagents. Many stained preparations are so dense that injected vessels and individual structures are obscured. Staining in haematoxylin, carmine, or acid fuchsin gives excellent results in the preparation of very young blastoderms. However, in the stages beyond fifty hours of incubation, owing to the multiplicity of structures and increased density of the tissues, the study of stained embryos is rendered very difficult for the beginner in embryology.

The following technique has certain advantages in that it renders the older stages more transparent and at the same time brings the individual structures into sharp delineation. It offers a variation from the usual laboratory methods and gives a preparation that is impervious to time, acid balsam, and the bleaching effect of sunlight.

The embryo is fixed in formalin (10 per cent) for twelve hours, preferably in a flat-bottomed shallow jar with just enough of the fixative to cover the embryo. This insures a flat preparation—a decided advantage in the case of chick blastoderms. Stain the embryo for two hours in haematoxylin and allow it to decolorize in a 5 or 10 per cent solution of NaOH. The time necessary for the decolorization depends upon the density of the stain, the

strength of the alkali used, and the age of the embryo. In the case of the younger stages, the weak alkali serves best and the time for decolorizing is short, from one to two hours. With the older embryos the stronger alkali and a longer period for decolorization is necessary. When all the blue color has disappeared and the preparation has become a light yellowish-brown tint, the decolorization is complete and the optimum density has been obtained. The blood vessels stand out as brown cords retaining their cell structure. The various organs are stained different shades of brown depending upon the density of their tissues. The whole preparation is translucent and resembles effects obtained by impregnation with silver.

The embryos are washed in several changes of distilled water, dehydrated in graded alcohols, cleared in xylol, and mounted in balsam. Greatest care should be observed, as the embryos are very fragile. Glass supports for the cover-glass must be used to prevent crushing of the specimen. The balsam should be as nearly colorless as possible. Under the binocular microscope with transmitted light, the various structures are readily made out. The mounts are not affected by acid in the balsam, nor are they bleached by sunlight; in fact, they improve with age.

## II. DISSECTION IN PARAFFIN

In embryological studies involving injections of the vascular tree, errors in interpretation are prone to occur because of the similarity of arteries to veins and the close interrelation of such structures. It is of great assistance to the illustrator as well as the research worker that the vessels to be demonstrated shall be isolated as far as possible from neighboring structures and that all overlying vessels be removed. In demonstrations for the class-room, it is of a decided advantage that the preparation be as little complicated as possible.

During a recent study on the origin of the pulmonary vessels in the chick, the study of whole mounts of injected embryos was difficult, in that the large cardinal veins and other structures obscured the delicate pulmonary capillaries beneath. In older

embryos this difficulty increased. I have had good results with a simple method of dissection that could be applied to other studies of embryonic circulation.

The injected chick is embedded in paraffin by the usual method and trimmed into a block with its broad surface parallel to the plane of the vessels to be demonstrated. In the case of the capillaries of the lung, this was the sagittal plane of the embryo. With gentle heat fix the block upon a glass slide. In a good light under the high power of a binocular microscope, holding a scapel lightly in the fingers of the right hand, shave off successive layers of paraffin until all the overlying vessels have been removed. A small window may be formed in this manner, exposing the desired structures and at the same time retaining the form of the embryo for localization and proportion. With a little practice and familiarity with the anatomy of the structures to be demonstrated, the result is in direct ratio to the ingenuity of the dissector. If desired, the block may be reversed upon the slide and the opposite side dissected in the same manner.

After these procedures have been carried out, the block is placed in xylol to dissolve the paraffin and mounted in balsam. In case of injections with dilute ink, the preparation is improved by staining to a light lavender tint with haematoxylin. This method eliminates a fluid medium, gives adequate fixation of the embryo during dissection, and protects the neighboring structures from injury during manipulation.





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Abstracted by Eben J. Carey, author. Marquette University  
Medical School, Milwaukee, Wisconsin.

Studies in the dynamics of histogenesis. Intermittent traction  
and contraction of differential growth, as a  
stimulus to myogenesis.

XI. The dynamics of the pectoralis major muscle tendon.

The origin and architecture of the pectoralis major muscle is intelligible only in relation to skeleton growth. At the beginning of the rotation of the fore limb there is no overlapping of the tendon of the pectoralis muscle. The rhythmic accelerated growth of the clavicle, sternum, vertebrae, ribs, and humerus draw out in intermittent traction the pectoralis major muscle along the successive resultants of the parallelogram of forces. The clavicle grows laterad, but the sternum cephalad, and the resultant is cephalolaterad from below, but caudolaterad from above as the limb is successively rotated ventrally. The overlapping of the pectoralis major tendon is the later resultant of the abduction and extension of the humerus caused by the synergists of the pectoralis major muscle.

## STUDIES IN THE DYNAMICS OF HISTOGENESIS. INTERMITTENT TRACTION AND CONTRACTION OF DIFFERENTIAL GROWTH, AS A STIMULUS TO MYOGENESIS

### XI. THE DYNAMICS OF THE PECTORALIS MAJOR TENDON

EBEN J. CAREY

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Milwaukee, Wisconsin*

SIX FIGURES

#### INTRODUCTION

Evidence was presented by the writer ('19, '20, '21) that tension of differential growth was the stimulating factor initiating the genesis of muscular tissue. The irritable primordial protoplasm responds to the initial adequate tension by contraction. The intermittent induction of the tractional stimulus is due to three factors, all of which are extrinsic to the zone of cells destined to be repeatedly stretched in the formation of muscle. These factors are: first, the dominant, epithelial growth in the tongue and intestine; second, the accumulation of fluid in the heart and bladder; third, the accelerated linear growth of the skeleton.

Differential growth connotes a dominant and a subdominant region. The former draws out the cells of the latter in tension. As regards the skeletal muscles, the subdominant area or region of retarded growth is the mesenchymal tissue that forms the muscles, the dominant zone or area of accelerated, linear growth, the skeletal elements. Muscular tissue is a sensitive indicator or tensiometer recording the degree of intermittent tension or work to which it is subjected by the extrinsic, dominating zone.

If these statements are facts and not fancy, then the origin and architecture of every type of muscle in the body, namely,

smooth, cardiac, and skeletal, may be defined in terms of intermittent tension. The genesis and growth of muscular tissues is a quantitative property of its work or exercise. This is due initially to the interaction of the intermittent tractional stimulus and the irritable protoplasm responding by contraction in areas of unequal growth.

#### DATA ON THE DYNAMICS OF THE PECTORALIS MAJOR MUSCLE

With these principles clearly in mind, the interpretation of figures 1 to 6 is relatively simple. These represent in diagram the later development of the pectoralis major muscle during that critical period of growth, the rotation of the fore-limb. The two zones of accelerated growth for our immediate attention are the sternal and clavicular areas. The sternum exerts its dominant growth activity in a longitudinal direction, whereas the clavicle is growing at an accelerated rate in a lateral direction. The resultant of these two growing forces may be resolved in a diagonal direction cephalolaterad by the parallelogram of forces at the region of the shoulder-joint. The humerus, adducted ventrad to the thorax, rotates inward at the shoulder-joint, due to the relationship it bears as a point of insertion for the premuscular tissue of the pectoralis major muscle. This premuscular tissue is subdominant in formative growth, and as a consequence it is drawn out in traction along the progressively formed resultants caudocephalad, resolved in the parallelogram of forces produced by the clavicular and sternal forces of dominant growth.

The rotation of the fore-limb is due to two factors: first, the accelerated longitudinal growth of the vertebrae and the transverse growth of the ribs are also involved; second, the subdominant growth of the embryonic musculature. The stretching of the mesenchyme because of differential growth leads to the reactive pull causing limb rotation. The dynamics involved in the tendon formation of the pectoralis major muscle is of main concern in this report.

The first active line of tug will be from its most distal point of origin to its most proximal point of insertion on the humerus. As soon as the humerus is adducted corresponding to this initial

muscular tug, successive lines of traction and resulting contraction are established caudocephalad at the origin (figs. 1 to 6, *a, b, c, d, e*) of the muscle and cephalocaudad at its insertion (figs. 1 to 6, *a', b', c', d', e'*). These progressive lines of muscular pull are produced by the accelerated growth of the related skeletal segments (clavicle, sternum, ribs, and vertebrae). As long as the humerus is free to rotate, the inevitable fore-limb rotation, in embryos 15 to 40 mm. in length, is the resultant. With the humerus completely adducted across the ventral aspect of the thorax, the embryonic muscular fasciculi are lineated along direct lines of adequate traction from origin to insertion. In this condition there is no twist in the tendon of the pectoralis major muscle. Those fibers arising from the clavicle and cephalic aspect of the sternum have a more distal insertion on the humerus, whereas those arising from the caudal aspect of the sternum and lower ribs have a more proximal insertion in the humerus (fig. 5). This relationship is the consequence of the successive induced lines of tractional stress in the premuscle mass by the rapidly growing skeletal segments.

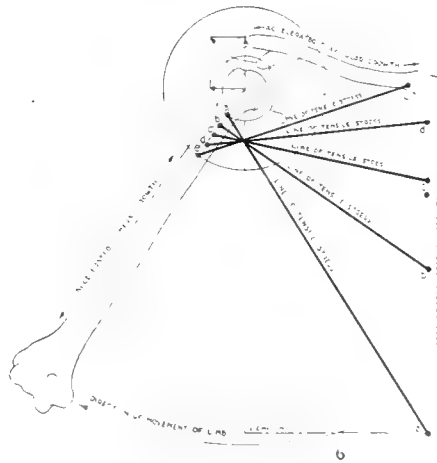
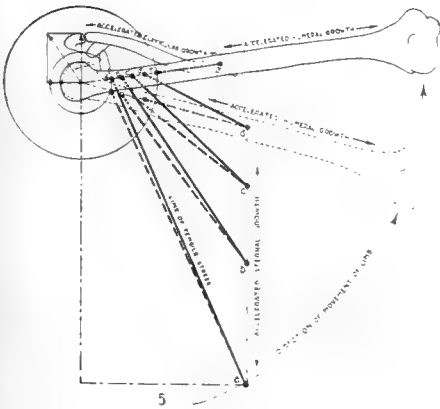
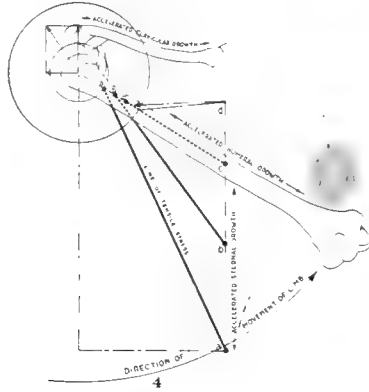
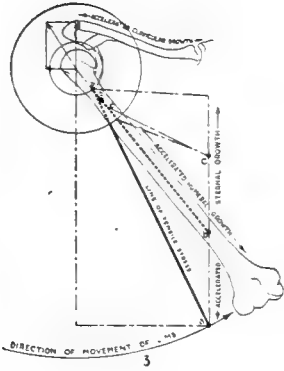
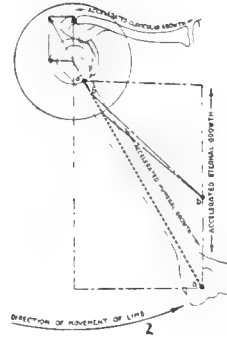
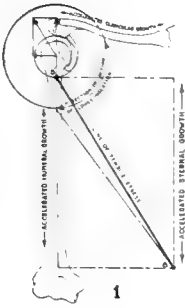
The limb is not fixed in this position by any means. There is active movement of the appendage due to muscular activity. The initial single sheet of the tendon acquires its overlapping characteristic with the interaction of traction and contraction manifested by the pectoralis major muscle. The synergists of the pectoralis major muscle abduct and extend the humerus in contrast to its former position of adduction and flexion. With this active interplay of opposing muscles the humerus is alternately in one position and then another. The abducting and extending activities of the synergists of the pectoralis major muscle cause the overlapping of its tendon (fig. 6). When the humerus is extended and drawn parallel to the side of the body or abducted the fibers arising from the clavicle and cephalic aspects of the sternum and upper ribs will inevitably assume a superficial relationship to those that arise from the caudal aspect of the sternum and lower ribs. The direction of the muscular fasciculi is determined by the lines of tensile stress induced in the premuscle mass by the accelerated growth of the related

skeletal segments. There is a tendency to untwist the overlapped condition of the pectoralis major tendon when the humerus is parallel to the clavicle in a flexed and ventro-adducted condition. But with the opposing activity, extension and abduction produced many times by the synergists, the architecture becomes fixed.

The successive appearance of new skeletal muscular components is directly determined by the progressive extension of the accelerated growing skeletal segments related to the muscle involved. One unfortunate term has been used in the literature of myogenesis, namely, '*muscular migration*.' Migration, in this sense, means an active movement on the part of the muscles from one region to another. The appearance of muscle fibers in

Figs. 1 to 6 This diagram first illustrates the humerus parallel to the lateral line of the thorax (fig. 1). The important skeletal zones of dominant accelerated growth are the sternum and clavicle. The growth of the ribs and vertebrae are also involved. The zone of subdominant growth is the pectoral premuscle mass. The sternal growth exerts its tension on the embryonic pectoralis major muscle in a longitudinal direction, that of clavicular growth in a lateral direction. The resultant of these two skeletal forces of growth may be resolved in a diagonal direction cephalolaterad by the parallelogram of forces at the region of the shoulder-joint. The fibers of the pectoralis major muscle during the period of fore-limb rotation progressively manifest their active tug from the distal to the proximal points of origin. This origination corresponds to the proximal-distal points of the insertion, as the humerus is adducted ventral to the thorax successive muscular fibers are formed.

Figs. 2 to 6 *a, b, c, d, and e* (origin); *a', b', c', d', and e'* (insertion). The fibers are stimulated by tension and assume their characteristic position because of the accelerated growth of the sternum, clavicle, and also ribs and vertebrae. When the humerus is parallel to the clavicle in a ventro-adducted position after the initial completion of fore-limb rotation, no overlapping of the tendon of the pectoralis major muscle is seen (fig. 5). When the synergists of the pectoralis major muscle extend the humerus and abduct it to a position parallel to the lateral line of the thorax, there is an inevitable overlapping of those fibers originating from the caudal aspect of the thorax and lower ribs by those which originate from the clavicle and upper ribs in the tendon of insertion (fig. 6). The latter group of fibers are consequently superficial in the tendon and have a more distal insertion on the humerus; the former are more deeply placed in the tendon and have a more proximal insertion. It is clearly apparent that accelerated skeletal growth not only induces the intermittent tension causing the genesis of the pectoralis major muscle, but the architecture is explicable only in terms of this accelerated skeletal growth plus the rotation and ventro-adduction of the humerus. The subsequent extension and abduction of the humerus cause the overlapping of the tendon of insertion of the pectoralis major muscle.



successive regions like the initial growth of the pectoralis major in a cephalocaudal manner cannot be defined as a migration. The apparent migration considered as due to an intrinsic activity of the skeletal muscles is in reality due to the rapid growth or extension of the related skeletal components. The consequent successive appearance of muscular fibers along lines of progressively formed adequate tension is caused by the dominant, accelerated skeletal growth of the components related to the area of subdominant growth of the mesenchyme. The induced lines of tensile stress appear successively in different areas due to the dominant accelerated, skeletal growth. All musculature is formed in situ along lines of adequate intermittent tensile stress; the subsequent apparent active shift on the part of the musculature is in reality due to the growth in length of the skeleton or increase in volume of the viscera where the diaphragm and other muscles are concerned. With the more definite form assumed by the skeleton during growth more definite direct lines of tensile stress are induced in the growing musculature. This causes a transposition of the lines of tensile stress; there is a consequent internal rearrangement of the muscular fibers along these later stress lines corresponding to that of the mature musculature. Certain of the embryonic muscle fibers not lineated in correspondence with the more permanent tensile stress lines will undergo degeneration. This physiological degeneration of muscle fibers will account for certain types of apparent 'Muscular migration.'

#### CONCLUSIONS

1. *Muscular tissue is a sensitive tensiometer or indicator of the intensity and rapidity of application of the tensile stresses induced in the premuscle masses by the extrinsic dominant zones of growth.*

2. The superficial position of the fibers of the pectoralis major muscle arising from the clavicle and upper ribs and inserting more distal on the humerus, and the deeper position of those fibers arising from the caudal aspect of the sternum and lower ribs inserting more proximal on the humerus are a resultant of the abduction and extension of the humerus caused by the syner-



gists of the pectoralis major muscle. This results in an overlapping of the tendinous insertion of the pectoralis major muscle.

3. In the inception of the fore-limb rotation there is no overlapping of the tendinous components of the pectoralis major, when the arm was successively flexed and ventro-abducted. Fore-limb rotation is caused by the accelerated growth of the clavicle, sternum, vertebrae, and ribs. This successive, accelerated growth draws out in traction the related muscles along the resultants of the parallelogram of forces. The muscular fibers are lineated along the adequate tensile stress lines induced in the premuscle masses. The clavicle grows laterad; the sternum cephalocaudad. The resultant humeral force is cephalolaterad, from below, but caudolaterad from above as the limb is successively rotated. The fibers of the pectoralis major are stimulated to formation in these successive positions. The origin and architecture of the pectoralis major is intelligible only in relation to skeletal growth and the intermittent tension induced by dominant skeletal growth.

4. When the arm originally assumed its position parallel and ventral to the clavicle in limb rotation the tendon of the pectoralis major was not overlapped. This overlapped condition became fixed when the arm was extended and abducted.

5. *The dynamics of myogenesis involving intermittent traction and contraction (work) of differential growth not only determines the genesis but the architectonics of the pectoralis major muscle. The genesis, growth, maturity, and hypertrophy of muscle is a quantitative property of its function or work. The genesis, growth, and hypertrophy of muscle is a direct index of an increase in the tension within definite limits. When the tension becomes relatively constant at maturity, the muscle remains relatively constant in size. With an increase in tension, after maturity is reached, there is increase in muscular work. Since Muscular Work = Tension times Contraction, an increase in tension within certain limits leads to increased function. This in turn produces muscular hypertrophy because of the increased metabolic rate.*

6. The overlapping of the tendon of the pectoralis major muscle is produced by the lateral replacement of the humerus in turn

caused by the synergists of the pectoralis muscle. The greater part of the pectoralis major muscle is formed caudocephalad during the period of the fore-limb rotation in human embryos 20 to 35 mm. in length. This myogenesis is the product of an intermittent tensional stimulus exerted in a cephalolateral direction as the resultant force of the lateral clavicle and cephalic sternal forces of growth.

I wish to thank Eugene Haug, consulting engineer, Milwaukee, and Leo Massopust, department artist, for their help in this work.



Abstracted by Edward F. Malone, author. University of Cincinnati.

#### Sharpening microtome knives.

This article discusses the nature of the edge of knives and the action of abrasive substances under different conditions. The preparation is described of hones sufficiently large to permit the knife to be sharpened without any portion projecting beyond the edge of the hone. The surface of the hone is leather glued to stone; the leather is then finished plane and treated with castor oil and grit. The methods of refining and testing grit are described at length, together with the proper care and use of the hones. Types of suitable honing backs are described and illustrated. The double concave knife is recommended, together with a large knife-holder adjustable for tilt. The method described is suited to the production of an edge uniformly good throughout its entire length rather than one of the very highest perfection.

## SHARPENING MICROTOME KNIVES

EDWARD F. MALONE

*Anatomical Laboratory of the University of Cincinnati*

FIVE FIGURES

In this article I propose to discuss the imperfections in design of microtome knives, the methods of making faulty knives serviceable, and especially the principles and methods of sharpening such knives as permit of being sharpened on a hone. That the edge of knives is usually unsatisfactory is a fact that permits of no discussion; the expression of more general complaint is due not to the absence of dissatisfaction, but to the belief that a good edge can be obtained only by an expert. While this belief is in general correct, the services of an expert are necessary only because he sharpens imperfect knives by methods requiring great skill. I propose to describe a method by which an excellent edge may be obtained without the use of great skill and which demands only the exercise of care and intelligence. The edge so obtained is not of the highest refinement, but is characterized by uniform excellence throughout its entire length.

The edge of a knife is formed by the intersection of two narrow plane surfaces, the cutting-facets; the base of the included wedge is continuous with the body, and each facet with a surface of the knife. The facets result through the formation by the hone of fine oblique parallel scratches, and these scratches result in a serration of the edge with the teeth directed towards the heel of the knife. To facilitate rapid cutting, the knife rests upon the hone only on its narrow cutting-facet and a second broader facet situated on the surface of the knife immediately adjoining the back; both of these facets or supporting surfaces lie in the same plane. To insure the knife's resting upon these two surfaces of limited area, each side is ground concave or else the back is elevated by the attachment of a special honing back.

In the process of sharpening it is thus evident that the edge is not formed directly, but is the product of the formation and development of the two cutting-facets, and that any defect which the edge possesses is the expression of some defect in one or both of the two intersecting planes by which the edge is formed. Since the cutting-facets are not smooth surfaces, but are marked by

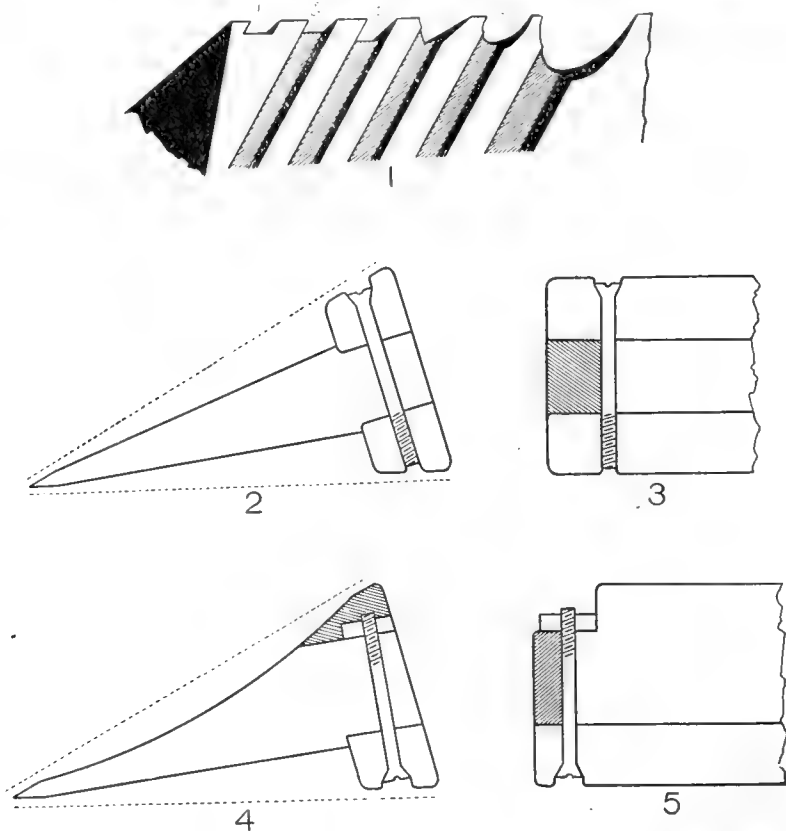


Fig. 1 Theoretical diagram drawn from wood model. From left to right a scratch intersecting with (1) near facet, (2) far facet, and (3) with another scratch; also scratches resulting in (4) a small V-shaped break, (5) a small U-shaped break, and (6) a true gap.

Figs. 2 and 3 Wedge-shaped knife and back. Shaded region in figure 3 represents slot; note rounded corners.

Figs. 4 and 5 Planoconcave knife and back. Shaded region in figure 5 represents slot.

oblique, parallel scratches, it follows that they cannot intersect in a smooth linear edge. The scratches upon both facets do not necessarily occur opposite one another and often cut the surface of the opposite facet after crossing the median plane. Since the scratches are parallel to the surface of the facets, their intersection with one another or with the opposite facet is at the same angle as that of the elevated portions of the facets. It is then evident that since it is impossible to form two perfectly plane surfaces intersecting to form a linear edge one should use every effort to approach this condition as completely as possible, to reduce the size of the scratches to a minimum while retaining the generally plane facets, without which a rounded edge results. An important reason for uniformly fine scratches is that when a deep one occurs the local pressure on the facet is tremendously increased, resulting not merely in a deeper serration, but also in an irregular breaking away of the steel near the edge which further deepens the gap (fig. 1).

The question arises as to why it is impossible to form two plane facets, polished free from even the finest scratches and intersecting in an edge free from serrations. Theoretically, this is possible by suitable stropping, but practically the lessening of the serrations is due largely to direct rounding off of the edge rather than to the primary removal of the scratches to which the serrations are due. In addition to the tendency to round off the edge, the strop is a source of further serious injury to the knife, the nature of which will be considered later.

Intersecting plane cutting-facets may be formed by means of a fine wheel or a fine plane hone. The wheel cuts the knife on the cutting-facet alone, while the plane hone cuts in addition a second facet adjoining the back of the knife. Since this second facet lies in a continuation of the plane of the cutting-facet, these two surfaces will stabilize the knife, and however often it be sharpened on a plane hone the angle and line of intersection of the cutting-facets will necessarily remain constant. Of course a knife could be thus ground on a wheel by passing the knife in a fixed plane tangent to the periphery of the rotating wheel, and the sharpening could be continued without interruption on a plane hone, since

a knife so ground would have on each side two plane facets lying in the same plane. It is hard to see why knives should not be so ground, but as a matter of fact they seldom are; usually the cutting-facets are ground without relation to the back, and this is unsatisfactory because the new edge is not sufficiently keen and cannot be improved on a plane hone and when dull must be re-ground on a wheel. This independence of cutting-facet and back is such that when the edge is placed in contact with a plane surface only one corner of the back will rest on that surface, the other corner failing to touch in many cases by as much as several millimeters; on the opposite side of the knife the opposite corner of the back is apt to fail to touch. Such a knife must be reshaped by grinding, and after the cutting-facets have been developed on a plane hone together with the secondary supporting facets, the cutting-facets should be narrowed by concave grinding. Of course a knife should be in this condition when purchased, but since they are usually not to be obtained in this condition they should be sent to be properly finished.

Accordingly, although the scratches made by a fine wheel are too coarse and cannot be reduced on a plane hone, the wheel is of great use in generally reshaping a knife, in removing large gaps, and in narrowing cutting-facets through concave grinding. The value of the wheel for this work lies in the uniformity and rapidity with which it cuts. On the other hand, the cutting-facets must be formed on a plane hone and the rate of cutting must be exceedingly slow, since a very coarse hone or the use of any external pressure will cause deep scratches and gaps. Since the steel must be removed very slowly by means of minute and uniform scratches on the cutting-facets, the process can be shortened only by keeping these facets narrow, thus reducing the amount of steel to be removed. By keeping the facets narrow a minute amount of steel can be delicately yet rapidly removed, and the keenness of the edge restored by a few strokes; theoretically to employ wide facets means lengthening the work from minutes to hours or even days, but practically it means failure, for in such a prolonged process accidents are almost sure to occur, thus indefinitely postponing completion. But there is a minimum



width of cutting-facet below which insufficient support is furnished, resulting in a rounded or broken edge. This minimum width depends upon the total pressure exerted upon that portion of the cutting-facet in contact at any instant with the hone, and since all external pressure must be avoided one needs only to consider pressure due to the weight of the knife. The most important factor is the angle formed by the cutting-facets, for as the back is elevated a greater proportion of the weight falls upon the cutting-facets. A thick, broad knife weighs more per unit of length, but this is counteracted by the shifting backwards of the center of gravity. A long knife on a narrow hone will be supported by less than the total surface of the cutting-facet, and even if the hone is so large as theoretically to support the entire surface of the facet, yet due to inequalities in the surface of the hone a long knife will exert greater local pressure than a short one, and coarse scratches with resulting gaps are always due to excessive local pressure. Practically I recommend a cutting-facet about  $\frac{1}{2}$  mm. in width, with a minimum width of  $\frac{1}{4}$  mm. and a maximum of 1 mm.; when the width exceeds the latter measurement, the cutting-facet should be narrowed to about one-half millimeter by removing its rear portion through concave grinding.

Narrow cutting-facets are obtained either by a double-concave grinding or, in the case of a wedge-shaped knife, by elevating the back through attaching a special honing back. The latter type of knife permits the facets to widen more rapidly than in the case of the double-concave knife and presents other difficulties to be considered later. The wedge-shaped knife is by some supposed to be more rigid than the double-concave type, but one should not overlook the fact that the wedge-shaped knife with attached honing back is usually sharpened at a more obtuse angle; to obtain the same angle of edge in knives of the same width the back of the double-concave knife should have the same thickness as that of the other type with its honing back attached. The rigidity of a knife depends almost exclusively upon the angle of the edge, which should be a little more obtuse than is required by the work which the knife has to do, and with equal angle of edge there can

be no essential difference in rigidity between a thin wedge-shaped knife and a thick wedge-shaped knife subsequently ground concave. Moreover, in comparing types of knives one is apt to overlook not only the angle of the edge, but those manipulations of the knife by which its tilt and slant are adjusted to the thickness of the section and the nature of the tissue. The only knife-holder worthy of consideration is one adjustable for tilt and large enough to hold a thick knife; the unfortunate replacement of the complete knife by the thin knife with removable back is encouraged by the small knife-holders usually supplied. While it may be possible that for certain kinds of work a plane surface is superior to one which is concave, yet this possible advantage can never compensate for an inferior edge, and a good edge is more easily obtained on a double-concave knife. Bearing in mind the fact that a double-concave knife should be ground from a wedge of steel whose angle, and therefore the angle of the finished edge, is suitable for the work required of it, I unhesitatingly recommend this type of knife. And I believe that the objections to it depend in large measure, if not entirely, upon the use of an edge of unsuitable angle and upon improper adjustment of the knife.

When the edge of a knife is too acute, the only way to increase its angle is to employ a special honing back. The honing backs in the market are useless, since they fail to stabilize the knife when the latter rests upon a cutting-facet, often shift position during honing, and cannot be replaced on the knife in the same position. I have had much experience with them and am convinced that those on the market are failures. This inferiority of design and manufacture of honing backs is not the fault of the manufacturer, but is due to the unexacting demands of their customers who have never considered honing in the light of a rational procedure, but one so closely allied to the supernatural as not to exclude the hope of obtaining a good edge by the formation of two rounded shifting facets determined by the unstable back. Moreover, these backs are supposed to fit any knife of a certain size and type, regardless of the individual peculiarities of knives due to warping, and often a back is not reserved for one particular knife, but is used on several. Until a more discriminating demand arises,

one cannot expect manufacturers to regrind their knives true after tempering and to supply each perfectly symmetrical knife with a honing back which will fit perfectly and can always be attached in exactly the same position. In fact, I recommend special honing backs only for expensive knives which one happens to possess and which cannot otherwise be suitably sharpened. No honing back can make a knife equal to a double-concave knife, and accordingly the latter is the only type which should be purchased. A honing back should not be attached to a double-concave knife; if the edge of such a knife is too acute, save the knife for suitable tissue; for using an oblique stroke, the increased acuteness of the effective angle of the edge will lessen the danger of injury, and under these conditions such a knife may be ideal. The two types of knives which must have honing backs are the wedge-shaped and plano-concave; the former should have an elevating strip on each side and the latter on the plane side only.

In connection with honing backs the following principles should be observed (figs. 2 to 5). Those portions of the knife and elevating strips which lie in contact should be ground perfectly plane and each strip should rest against a slight shoulder on the knife; this insures a perfect fit necessary for stability and accuracy in reassembling. The two surfaces of the knife in contact with the strips are best ground parallel to one another to insure accuracy in drilling the strips as they can be drilled while clamped together. The knife cannot be drilled and therefore the screws are made to lie in narrow slots, at each end of the knife, ground perpendicular to its two parallel surfaces; if a third screw seems advisable, a slot could be ground for it in the middle of the back of the knife. The elevating strips must be drilled and tapped and therefore of soft steel or iron, the tendency to wear away rapidly being balanced by the relatively large surface. These strips should be of sufficient thickness to give, after they are reground, the desired angle. Due to the grinding away of the surfaces of the knife, these strips are thick enough to avoid vibration and bending and to allow countersinking of the machine screws. The machine screws should have a diameter of  $\frac{1}{8}$  inch and be flat-headed, since this type can be more accurately countersunk. The slots

should be so deep that the head of the screw will be removed from the end of the knife by at least 1 mm. When attached the elevating strips should be so ground that their surfaces lie in the same plane as the edge, and after the formation on a hone of the cutting-facets the latter should be narrowed by lightly grinding away their posterior border. All edges and corners are rounded off to protect the hone. Of course each knife must have its own back and each should be identified by number, and the pieces marked to insure proper assembling. In case of the planoconcave knife, the back of the concave side is ground parallel to the plane surface, but only at each end. While I have as yet never had the equipment to make such a back, I have prepared much cruder ones which are satisfactory, and accordingly do not hesitate to recommend the back described above. As previously stated, such a back will often make an expensive knife usable, but in ordering new knives double-concave knives of proper angle should be specified.

Assuming that we have a double-concave knife or a knife with a honing back which remains rigidly in place and can always be replaced in exactly the same position, we may now consider hones and methods of sharpening. From the nature of the cutting-facets it follows that a hone should be plane and should remain so, and that if it is large enough to permit one to keep the entire knife always upon the hone the process of keeping the facets plane will be made more accurate and much easier to accomplish. For if the hone is plane and cuts finely and if the entire length of the facet rests upon the hone little skill is required to produce a good edge. Assuming the size of the desired hone to be 1 foot by 2, it is evidently impossible to secure a hone of the usual type in this size, and artificial abrasives which can be made into a stone are too coarse.

At first I tried sharpening on plate glass with fine carborundum powder and water, and after several years found why this method can never be successful. The facets appear very evenly and finely frosted, but partial polishing reveals the presence of deep scratches and numerous pits extending deep into the steel. Unless of extreme fineness a freely moveable grit between two moving

hard surfaces will always cause the formation of pits and gashes under cover of the delicate surface finish. This method has been successfully used by Funck (*Zeit. f. wiss. Mik.*, 1910), who employs an extremely fine grit, thus limiting the method to the formation of the final edge; however, the danger from the accidental presence of even relatively fine foreign grit is always present. Reflecting upon the difference in behavior between a movable grit and the fixed grit of a stone, it occurred to me that the complete fixation of the grit was far from ideal; for instance, the tendency of the stone to pull out particles of steel and the danger of injuring the edge on the hard stone in case of a slight accident. As a result I decided to try the effect of partial fixation of the grit, embedding it in a surface which would not hold it firmly against excessive resistance, and covering the surface with a thin layer of grit which would pack into a self-leveling abrasive surface unyielding under the weight of the knife. The material selected was leather treated with castor oil and has justified all expectations.

The leather surface must have a rigid support which will not warp, and accordingly wood cannot be used. I use any soft stone which can be easily cut with a saw, ground plane, and the corners and edges finished with a rasp, while sufficiently porous to hold glue. The upper surface of the stone is ground plane after well rounding off the corners and edges to prevent breaking. Three such stones should be prepared, since a coarse, a medium, and a fine hone are necessary. The two finer hones should measure 8 inches by 24, while the coarse hone should measure 12 by 24 inches. Before handling the leather all grit must be removed from the stone, hands, clothing, and surroundings, since any grit once embedded in leather can never be removed. For leather I use a good grade of cowhide of uniform thickness and texture. In clean surroundings the leather is cut to the proper size, leaving a margin of stone at least 1 cm. wide and the corners of the leather cut off round to correspond to those of the stone, since sharp corners tend to become detached. A plane surface is selected of suitable size and such a nature as to permit the use of large clamps, and is covered with a piece of clean paper to prevent

the leather from sticking to the surface beneath the paper. The leather is placed dressed side down upon another piece of paper in a different location and both the leather and the surface of stone given a coat of hot glue of a good grade; from now on one must work quickly. The two coated surfaces are placed in contact and the leather smoothed out, pressing out firmly the excess of glue which is wiped off with a damp cloth, care being taken to remove any glue on the upper surface of the leather. The hone is then placed carefully, leather surface down, on the paper-covered surface prepared for it and the clamps carefully and uniformly tightened to counteract the tendency of the leather to shift its position.

After twenty-four hours the clamps are removed and the leather surface carefully dressed with a plane. The plane should be at least 15 inches in length, very sharp and free from gaps. The plane must not cut deeply, since this might cause the leather to tear or crack, and should be moved in all directions, especially along the diagonals, frequently turning the hone end for end, so as to insure a plane surface. When the surface is plane the edges and corners are slightly beveled and the surface finished by lightly shaving with a sharp microtome knife. This knife must lie flat on a plane surface and is lightly moved edge first in different directions in such a manner as to render the surface plane and evenly finished. The leather surface should have a fine velvet finish and is now ready for the oil and grit. When not in use each hone should be covered with its own properly labeled dust-proof top of wood or cardboard.

The leather surface of each hone is treated with castor oil, which should be uniformly distributed and thoroughly rubbed in. More oil is added and upon the addition of the appropriate grit the resulting paste is rubbed into the leather; fresh grit is added from time to time. The rubbing should be vigorous and rags or paper should be used. As the paste becomes thick the velvet surface of the leather begins to come away with the paste and the leather becomes smoother and firmer. This thick paste fills the pores of the leather, and when the hone is in use will form a thin firm layer over the leather which should be covered in turn by a

very thin layer of dry grit. Do not add too much oil, since the excess must be removed and this is tedious and requires the use of much grit. After a day or two when the oil has soaked in the excess of oil is removed from the leather by repeated and vigorous rubbing with many clean pieces of paper toweling. This process should be thoroughly carried out so as to avoid the necessity of too frequent repetition. Before use the leather surface is brushed off, rubbed with the palm of the hand to free it from foreign particles, and a little grit added, which is rubbed in by hand and smoothed with the palm. Beginning from the time that the grit is first rubbed into the hone, each hone should rest on a separate table in surroundings free from coarse grit and should have its own dust-proof top. Since the surface is always smoothed with the palm, the hands and finger-nails must be carefully cleaned, especially when one is to use the finer hones, and one should always work with bare forearms. Honing backs must be removed from knives and each carefully washed with soap and water and then dried, paying especial attention to recesses in which grit may collect; this is one of the disadvantages of such backs. An ideal source of contamination is the greasy castor oil bottle and its cork, which should be protected from all grit and carefully cleaned before using. The necessity of care in protecting the fine hones is imperative, since coarser grit once imbedded can never be removed; it can, however, by repeated cleaning be rubbed into the leather and kept covered with fine grit.

The nature of the surface of the leather hones must be carefully considered; the surfaces of the three hones differ only in that the finer the grit the more delicately it cuts and the more readily it packs into a compact layer. The addition of castor oil to the leather changes the hard leather with superficial velvet finish, a finish which would round off the edge, into a dense, sticky, homogeneous mass. Thus the velvet surface is either rubbed off or else incorporated with the rest of the leather, and the softened pliable leather is more receptive to the grit. Without the presence of grit oiled leather will cause a knife to adhere tightly and is absolutely useless. This tendency to cause the knife to adhere continues to reappear to a slight extent after the grit is added,

and when slight may be overcome by rubbing up the grit and redistributing it with the palm of the hand. But in new hones frequent rubbing off of the excess of oil with paper is necessary. The carborundum grit fills up the pores of the leather and renders it firmer, and while rouge on oiled leather will cause the knife to adhere hopelessly, carborundum grit if properly manipulated will form a surface over which the knife glides without any pressure other than that due to its own weight. The dry grit should form, above the firm paste, a definite layer, but one whose thickness is almost inappreciable. When gently roughened up with the palm, the grit, as the knife passes over it back foremost, fills in any irregularities in the leather and automatically forms a plane surface. The leather is inelastic, but is readily dented through pressure exerted by a sharp object, such as a corner of the knife or a finger-nail. These marks may be removed by rubbing or forcibly ironed out by any object having a straight rounded edge, such as the edge formed by the back and main surface of the knife, but such depressions are harmless unless excessive and soon become filled with dense paste or dry grit.

This soft, homogeneous consistency of the leather is the source of advantages not found in case of a stone surface. Under the weight of the knife the leather is absolutely unyielding, the surface being as dense as the rest of the leather, and in this respect it is equal to a stone surface, while in other respects it is superior. The leather does not cause the knife to vibrate, it always remains plane, and in case of slight accidents the soft leather will not hold the grit firmly, thus avoiding great injury to the knife. Such accidents as slightly elevating the back of the knife, the presence of foreign particles on the hone, or striking the edge of the knife against the edge of the hone, while very serious in case of a stone surface, cause relatively little damage in case of the leather hone. In other words, oiled leather is as harmless a substance as will support a knife without yielding under its weight, while the particles of embedded grit are capable merely of individual action which is limited, in the event of unduly increased local or general pressure, by the ability of the particles of grit to give way; whereas a stone acts both through its rigidly fixed



individual particles and also through the unyielding mass which these particles form. Moreover, unlike stone or plate-glass, leather cannot give off any hard particles such as are a source of constant danger in using a hard surface. The ease and certainty with which a good edge is obtained on a leather hone is the result of avoiding or reducing to a minimum the effects of those frequent slight accidents which are often undetected.

The nature of the grit and the method of refining it will now be considered. The entire support of the cutting-facet of the knife is equal to the combined support of all the individual particles of grit with which the facet is in contact. In the case of coarse grit, the tops of these particles are relatively far apart, thus offering relatively less total support to the facet and thereby causing the facet to exert a relatively greater local pressure on each particle; moreover, the greater height of these particles permits them to cut more deeply into the facet before the latter receives additional support from deeper-lying particles. In the case of a fine grit, the total area of contact with the facet is greater while this increased surface of contact is formed by a much larger number of particles, and consequently the local pressure on each particle is decreased, thus causing them to cut less deeply. Grit cuts most rapidly when used upon a hard surface, such as a plate of glass, less rapidly when held together by a binding substance, as in the case of a stone (except when particles of grit become dislodged), and least rapidly when used upon a surface which will allow the particles to sink in, thus tending to equalize local differences in pressure. A large particle of grit will cause much less damage when used with other particles of the same size than when used with finer particles, since in the latter case the entire support of the facet is furnished by the single large particle which extends above the general surface of the fine grit; moreover, the fine grit will form a delicate edge so that under these conditions a large particle will not only cause a deeper scratch, but will break away the edge to a greater extent. Isolated coarse scratches with their accompanying gaps are merely the result of abnormal local pressure, of local rapid cutting during a stage of honing wherein the general rate is slow; whereas during a stage of honing character-

ized by general rapid cutting the roughness of the edge must be considered normal. Accordingly, during any stage of honing the size of the scratches should be nearly constant, larger or smaller according to the rate at which the steel is removed. In other words, however coarse or fine the grit, it will cut with maximum rapidity and with minimum injury when its particles are of uniform size.

Carborundum grit should be used, since in addition to its extreme hardness it spreads into a smooth layer without the tendency to form isolated lumps; a 5-pound can of 'FF' and also of the 'Sixty minute' powder should be procured. It is absolutely essential to refine these commercial powders, and when refined the coarse or FF powder is used for the coarse hone, while the Sixty minute yields, after grinding it into a finer powder, two grades of powders for the medium and fine hones, respectively. The refining is carried out by settling in water and the result controlled under the microscope with the aid of a micrometer eyepiece. Under each of two conditions some of the grit will fail to settle in water, for dry grit will form a surface film over the water and air bubbles will carry a film of grit upwards; these circumstances, while of no importance during the elimination of particles too small to be of use, involved most serious consequences when the particles to be eliminated are too coarse for use. Accordingly, a little of the dry powder should be placed in a mortar, and when thoroughly mixed with water it is poured into a liter measuring cup or pitcher, which when filled is caused to overflow by the addition of water and the floating grit and air bubbles brushed off. During subsequent operations, whenever coarse grit is to be eliminated, the bottles should be filled to overflowing immediately before allowing contents to settle, and the surface grit floated off before introducing the siphon; moreover, as the surface falls in the bottle it may regain a film of grit from that adherent to the sides so that the upper centimeter of the contents should never be siphoned off. One must remember that the position of a particle of grit which has been allowed to settle for a definite time depends not only upon its weight, but also upon the point from which it started; therefore the separation can be

accomplished only by repeated settling and siphoning with the use of many changes of water. All particles above a certain size will settle each time, but will be mixed with many useful smaller particles, and after all the latter have been recovered by repeated settling all the useful particles can be made to settle each time, but must be washed free by repeated settling from those particles which are too small to use. Another source of contamination is due to the disturbing of the settled grit through the current set up by the siphon, so the end of the tube should never closely approach the layer of settled grit and the stronger the stream the greater should be the distance; this is naturally of greater importance in the case of the finer grades. Finally the grit should be dried at room temperature, since if dried over a flame it will unite into dense masses which will not disintegrate upon rubbing.

The FF carborundum powder contains relatively few particles too coarse to use upon the coarse hone, but many which are too fine, including a considerable amount of what appears to be rouge or else mud; on account of this contamination the finer particles should not be saved for the finer hones. In preparing the grit for the coarse hone I reject all particles large enough to settle 10 or 12 inches in one-half minute, and reject all those too small to settle 5 or 6 inches in one minute. It is best to begin by removing the excessively small particles by thoroughly rubbing up the grit in a liter cup of water, allowing it to settle one minute, and pouring off half the contents; this is repeated until the water comes off clear. After this preliminary cleansing, the grit is poured into a bottle 12 or 15 inches high holding at least a gallon, and by repeated settling all grit siphoned off except that too coarse to use. Each time the contents is thoroughly mixed without undue formation of bubbles, the bottle filled to overflowing and the contents allowed to settle for one-half minute, after which the surface layer of grit and bubbles is again brushed off and the rubber tube introduced so that its inlet reaches within about 3 inches of the bottom. The contents is siphoned into a second gallon bottle, and as the pressure falls the inlet can be lowered, but never nearer to the settled grit than 1 inch. Since

the suspension is opaque, the tube should be so marked by adhesive tape that one can tell the position of its inlet; after each operation the tube should be washed inside and out. The above operation is repeated until all useful grit is removed from the first bottle, and since it is convenient to collect all of this useful grit in one bottle, each time this second bottle is filled its contents is mixed, allowed to settle two minutes, and siphoned off in the sink. After all the useful grit has been collected in the second bottle, the smaller particles must be eliminated by allowing to settle one minute and siphoning off into sink the upper half of contents, keeping inlet of siphon at a depth of 5 or 6 inches; after mixing the rest of contents and settling for the same time, the rest of the suspension is siphoned off. This is repeated until the water comes away clear. The grit should be well mixed and a sample examined under the microscope. The average size of particles should be 35 to 40  $\mu$ ; there should be few smaller than 15  $\mu$ , although some as small as 2  $\mu$  will appear. Particles 75  $\mu$  in length should not measure over 50 in width, but a few narrow ones may occur with a length of 150  $\mu$ . Since the particles are separated according to weight and since their shape varies, it is impossible to prevent a considerable variation in their long diameter. In this grade of grit the danger is in the presence of too many fine particles below 15 or 20  $\mu$  in diameter. After testing, the grit is filtered, washed with distilled water and preferably alcohol, and spread out to dry without the use of heat. It is kept in a properly labeled bottle with a cork stopper; a glass stopper must not be used.

The Sixty minute carborundum powder from which the medium and fine grades of grit are prepared is much cleaner than the FF powder and contains few particles too small to use, while the majority have to be ground still smaller. At first it is best to remove from the powder all particles small enough to use and then grind up the larger ones, since grinding is liable to eliminate too many of the size suitable for the medium grade of grit. The dry grit should be most thoroughly rubbed up with water until it goes into suspension, and great care must be taken to avoid surface films and films upon air bubbles; the inlet of the siphon

must be kept well away from the settled grit, since the latter is easily disturbed. The upper limit for the medium grade is a fall of 6 inches in two minutes and the lower limit an equal distance in ten minutes; but the latter time could probably with advantage be reduced from ten minutes to five or six, thus removing some of the finest particles. The upper limit of the fine grade is a fall of 6 inches in half an hour and the lower limit 12 inches in eighteen hours; but beyond doubt the grit would be improved by moving both time limits nearer together with an upper limit of one hour and a lower of six to eight hours. The settling process, filtration, and drying are carried out as previously described, remembering that whenever coarse grit is to be eliminated the bottle must be filled to overflowing before introducing the siphon and that the inlet of the siphon should never extend more than half-way to the bottom of the bottle. A large portion of the grit will be too coarse and must be ground before refining. A little grit is mixed with water to form a paste and ground between two glass plates which are then washed in a large pan of water, and the process repeated until the water is very milky; the added glass particles cut rapidly and are not objectionable. The milky water is poured into a large bottle and the coarse grit remaining in the pan saved for grinding. From this milky suspension the two grades of grit are prepared, first eliminating grit too coarse for the medium grade and then that which is too fine; from the latter the fine grade of grit is prepared. On testing the medium grade the largest particles should not measure more than  $30\ \mu$  in diameter and there should be few below 8, while the average is about  $17\ \mu$ ; as previously stated, I advise changing the lower limit from ten minutes to five or six. The fine grade should have no particle over  $13\ \mu$ ; the smallest are about  $0.5\ \mu$ , and the average about 4. This variation is certainly too great, and I strongly advise changing the upper limit from half an hour to one hour, and the lower limit from eighteen hours to six or eight hours.

The general method of using the hones will next be described, followed by additional observations in the case of each hone. The fundamental fact to remember is that the knife should always

rest with merely its own weight upon the hone; all external pressure, however tempting, should be avoided, and the other source of pressure, namely adhesion, must be as carefully avoided through the proper care of the surface of the hone. The strokes employed are the same as in stropping, oblique strokes on alternate sides with the back moving in advance of the edge and the toe in advance of the heel; a handle must not be used nor should any portion of the knife project beyond the edge of the hone. Before each stroke the surface of the hone is lightly roughened up with the palm of the hand; this prevents the grit from packing into a hard layer and maintains a thin film of loose grit on the surface. Such a surface film of loose grit prevents adhesion of the knife to the hone and causes the latter to cut more rapidly. If sticky patches of grit appear, they can often be removed by vigorous rubbing of the entire surface of the hone with the hand so as to mix the oily grit with the dry, but at times the excess of oil must be removed by rubbing off the sticky paste with clean paper, after which fresh grit is applied and smoothed out with the palm. To prevent the formation of gaps through breaking the thin edge, the latter should, except in the case of the fine hone, be kept rounded and smooth. This is accomplished by lightly drawing the edge several times over the ball of the thumb covered with grit; the edge is removed before it can enter the epidermis. The edge is restored by about five double strokes on the hone, and accordingly the dulling should be repeated often, the frequency of the process depending upon the thoroughness with which it has been carried out.

The coarse hone is used when gaps exist, when the facets fail to intersect, or in any condition where much steel has to be removed, and when these defects have disappeared nothing is to be gained by longer use of this hone. The edge should be kept well rounded and smooth as just described, but if any doubt exists as to the intersection of the facets throughout their entire length, this dulling process should be discontinued until the edge along its entire length will cut a coarse hair; thereafter the dulling operation is resumed. Since the coarse hone should cut rapidly, the surface layer of loose grit should be thick, about 1 mm. in

thickness, and should be brushed off and replaced with fresh grit when it becomes oily and broken up into finer particles, thus slowing its cutting. Near the end of the operation the excess of grit should be removed, leaving a surface layer of loose grit about  $\frac{1}{4}$  mm. in depth, and this grit should not be replaced by fresh: in this manner the facets will be more nearly plane and the knife in better condition to transfer to the medium hone.

The medium hone is used after the coarse and also to remove gaps which have appeared in the finished edge when these do not exceed a size barely visible to the unaided eye. As in the case of the coarse hone, it should have a surface layer of loose grit lightly roughened up by hand before each stroke. But since at this stage rapid cutting is of less importance than accuracy, this surface layer should be thin, about  $\frac{1}{8}$  mm. in depth, and since the grit is relatively valuable it should be renewed only when necessary. Upon the gradual intermixture with oil, this grit will form a sticky paste more readily than the coarse grit, and accordingly more attention is necessary to maintain the thin surface film of loose grit so essential to prevent adhesion between the knife and hone. Greater care against contamination is necessary and before using the hone or when it otherwise appears advisable, the surface should be blown off and then lightly brushed off by hand, adding fresh grit when necessary. With the foregoing modifications the procedure is the same as usual, the knife moving obliquely under its own weight alone, back forward and toe in advance of heel, reversing sides and direction after each stroke, the surface grit lightly disturbed before each stroke, and the edge kept rounded and smooth. A medium polish appears on the facets, and the edge if not purposely dulled, as it should be at least at the beginning and the termination of the process, becomes very keen, but shows slight microscopic defects.

The fine hone is used after the medium hone and also before and after employing the knife for cutting. Its fine grit has the tendency to pack into a firm smooth layer which may cause the knife to adhere and its edge to break, and consequently a little fresh grit should be added after about twenty double strokes.

The fresh grit has the feel of talcum and should be frequently added in small quantities forming above the firm smooth layer of grit a film about as thick as a rather heavy film of talcum. If the hone is thus kept with a surface which has the feel of a film of talcum it will cut relatively fast, and the knife will glide over it with the greatest ease, showing no tendency to adhere. The method of sharpening is as usual, the surface layer being very lightly disturbed before each stroke, and all pressure on the knife as well as all careless manipulation being carefully avoided. This stage of honing should be carried out with deliberation, keeping clearly in mind that by the delicate removal of minute films of steel one is bringing to complete intersection two polished and perfectly plane facets; with such an ideal in mind any position of the knife which allows the facet to rest upon the hone in any manner other than one of light yet perfect contact will be recognized as the result of stupid carelessness in the manipulation of the knife. At first the edge should be kept dull as described, but later of course it must be allowed to reach its maximum keenness. After the imperfections due to the medium hone have been removed, the final finish is given to the edge by allowing the surface grit to pack somewhat, slightly disturbing it after every second double stroke, instead of after every single stroke. This gives an edge which is keener and more highly polished, but the tendency of the knife to adhere must not be allowed to go too far. It is evident that very careful refining of the grit is essential, for the perfection of the finished edge depends upon the nature of the grit.

The resulting edge is very keen, with highly polished facets, and should be used without attempting to improve it on the usual leather strop. Oiled leather without grit will cause the knife to adhere firmly, and if it is not oiled the surface is very apt to have rough places. Such rough places on soft leather will round off the edge, while on hard leather they will cause deep scratches and gaps to an extent almost unbelievable. A great deal of damage is done to knives by stropping, the nature of the injury depending upon whether the surface is sticky, fuzzy, or hard and rough. Even if a strop is hard and smooth it offers a surface upon which



a piece of lint or a particle of grit will do the maximum damage to the knife.

The edge should be tested by inspection under the microscope and by the manner in which it will cut a hair. Both of these tests are necessary, since in my opinion the keenness of the edge cannot be determined by inspection, which, however, gives most reliable information as to uniformity; while the manner of cutting a hair will give reliable information as to the relative keenness of large portions of the edge, but fails to indicate the extent or even the presence of sharply localized defects. The microscopic inspection is carried out by reflected light under the 16 mm. lens. A firm support, such as a book or a board, is placed on the stage and covered with a piece of clean paper upon which the knife is placed flat with the edge directly facing the light. The knife must not be moved over the paper, but only by moving its support over the stage; the light should always fall directly upon the cutting-facet from in front and above. In testing for keenness one should remember that while a rough edge will cut a coarse hair more readily than a fine hair, the reverse is true of a finely serrated edge, since it cuts a soft delicate hair with ease, but a coarse hard one only with the aid of pressure. An edge finished on the fine hone should throughout its entire length instantly sever a fine hair with extreme suddenness and smoothness. If the edge throughout its length meets this test of keenness and under low power of the microscope reveals no appreciable inequalities it is thoroughly serviceable. In fact, an edge which shows a few very minute gaps is for most purposes usable, since the loose grit tends to polish and round off and thus lessen such slight defects, an advantage especially apparent when the knife is used in the oblique position which permits such rounded and polished depressions to remain practically inactive.

A knowledge of the principles which underlie the process of sharpening is essential to success, and I have attempted to make their consideration sufficiently general to apply to all methods employing a plane hone. Since the principles and methods have been discussed somewhat at length, the impression may result

that the method described must of necessity be complicated. But the complexity lies in the operation of a large hone with partially fixed grit, a hone which through its lack of simplicity of operation automatically removes many sources of danger and thus permits the operator to attain success with certainty and with a minimum of skill. While the requisite skill has been greatly reduced, the delicacy of the edge is such that no method will assure good results in the absence of intelligence and care. Accordingly, it has appeared desirable to present a thorough discussion of the principles, aims, procedures, and errors, since their appreciation must always form the basis of intelligent effort.



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A note on the elastic membrane of the bronchial tree of mammals, with an interpretation of its functional significance

The network of longitudinal elastic fibers which forms a membrane in the tunica propria of the entire bronchial tree of mammals may be dissected off en masse from the fresh trachea and larger bronchi, and the specimen so obtained may be stained, cleared, and mounted. Stout in the trachea, and especially in the larger bronchi, this elastic membrane becomes thin and delicate in the terminal twigs, where it unites with the elastic tissue of the air chambers. Considered in toto, its form reproduces that of the entire bronchial tree. The limbs and trunk of the bronchial tree constitute a system of extension and contraction axes for the movements of expansion and contraction of the lung. The filling and emptying of any considerable area of lung is inseparable from the stretching and contraction of the airway. The elastic membrane is by far the most efficient part of the recoil mechanism of the bronchial tree, and is a very important, if not the most important, part of the recoil mechanism of the entire lung. Downward shifting of the lower part of the lung is necessary to free expansion of the upper part, and is associated with downward movement of the lung root and stretching of the bronchi and trachea. Pathological changes which tend to immobilize the lung roots or stiffen the bronchial tree will impair normal lung function, particularly in the upper lobe, and such changes may go far to account for the susceptibility of this part of the lung to tuberculosis.

## A NOTE ON THE ELASTIC MEMBRANE OF THE BRONCHIAL TREE OF MAMMALS, WITH AN INTERPRETATION OF ITS FUNCTIONAL SIGNIFICANCE

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### CURRENT KNOWLEDGE OF THE ELASTIC MEMBRANE

Attention has often been called to the fibers of elastic tissue which course in a longitudinal direction beneath the epithelium of the trachea and bronchi in all the higher animals, but so far as I am aware no one has sufficiently emphasized their prominence and importance in respiration. A typical text-book description is that of Jordan ('20, p. 302), who, writing on the trachea, reports as follows:

The tunica propria includes a thin inner layer of connective tissue which is richly supplied with small blood-vessels and infiltrated by many lymphocytes, and an outer layer of elastic tissue most of whose fibers are longitudinally disposed. The elastic layer begins in the region of the vocal cords in the larynx and is continuous below with the similar layer of the bronchial mucous membrane. Elastic fibers are more numerous in the trachea of the lower mammals than in that of man. A muscularis mucosa, a characteristic structure of the mucosa of the digestive tube, is lacking in the trachea. The elastic membrane occupies the position held by the muscularis mucosa in other organs.

Farther on (p. 309), speaking of the respiratory bronchioles, Jordan says:

The elastic fibers, derived from the elastic layer of the bronchioles, pass over to the alveolar walls in which they form a delicate network.

Other text-books (Landois, '92; Koelliker, '99; Luciana, '11, et al.) describe these fibers at greater or lesser length. The most comprehensive description of them, from the point of view of

comparative anatomy, is found in the well-known work of Oppel ('05), in which it appears that, though they are present in the respiratory tract of birds, and are even represented rather indefinitely in amphibians and reptiles, they reach their highest development in mammals. Within the mammalian group, too, they present some variation, but in all they show the same fundamental plan. So constant are they, and so conspicuous, that it is impossible to escape the conclusion that they must play a very important rôle in the physiology of the respiratory tract. The vascular subepithelial layer, rich in lymphocytes, is described by most authors and may be merged with the elastic membrane proper in some animals. The muscle of the bronchial tree is reported as situated just outside of the elastic membrane, the fibers being mainly circular in disposition, while the fibers of the elastic membrane are longitudinal. Beyond the muscle, again, are the cartilages of the airway, followed by a layer of adventitia. In the adventitia are numerous elastic fibers, but these do not form a definite membrane, and in direction they are irregular.

#### NOMENCLATURE

There appears to be no generally accepted term of designation for this system of fibers. Jordan, as we have noted, refers to this layer of elastic fibers collectively as the 'elastic layer' or 'elastic membrane.' Among the names which are used by the German writers are 'subepitheliale elastische Faserschicht,' 'elastische Längsfaserschicht,' and 'subepitheliale Schicht elastischer Fasern.' Since the fibers make up a system extending from the larynx to the outermost terminations of the bronchial tree, I shall refer to the entire system as the 'elastic membrane of the bronchial tree,' and shall consider it as extending from the lower end of the larynx, through the trachea, larger and smaller bronchi, and bronchioli to become continuous with the elastic fibers of the respiratory tissue of the lung. Often, for brevity, I shall refer to it merely as the 'elastic membrane.'

In the following pages I have limited myself to a consideration of these fibers as they occur in the higher mammals only, and particularly as they are found in the cat, dog, pig, and sheep.

## APPEARANCE IN THE FRESH CONDITION

The elastic membrane of the trachea and bronchi may be readily observed in the fresh specimen with the naked eye. In the respiratory tract of the pig, following the dissecting away of the lung substance, the slitting open of the airway, and the careful sponging away of mucus, the elastic membrane, characteristically creamy white in color, may be seen shining through the epithelial layer. In the trachea, by close examination, the interwoven appearance of the fibers is evident, the membrane showing as a plexiform mass or network, with meshes running in a longitudinal direction. In the larger bronchi the appearance is that of longitudinal whitish-yellow streaks, raised into folds, where the elastic fibers are concentrated into dense strands or trabeculae, as many writers have pointed out. As the limbs of the tree are followed out to the smaller branches, the fibers of elastic tissue become less and less conspicuous.

## APPEARANCE OF THE FRESH ISOLATED MEMBRANE

In the fresh lung of the pig the operation was tried of stripping off the elastic membrane entire. After clearing away the lung tissue and opening the main tubes, a beginning was made at the upper end of the trachea. It was found to be possible to dissect away, with the aid of forceps and a small scalpel, the mucosa containing the elastic membrane as its densest constituent. The stripping of the trachea in this manner was quite easily accomplished. As progress was made down the bronchi, however, difficulties were encountered and lacerations of the membrane were frequent. The main bronchial stems were followed, the membrane being cut across at the smaller branches. It was found to be quite a difficult task to make a clean stripping of the elastic membrane in the medium-sized bronchi, and in the smaller ones it was impossible, since the membrane became very thin and delicate. However, enough of the system was obtained in this way to make a good preparation.

When spread out there appeared a Y-shaped band of elastic tissue, the diverging limbs of the fork rapidly diminishing in width and density, and showing perforations for the branches

of the bronchi, as well as a ragged appearance along the edges. The tracheal portion was practically intact. When stretched and allowed to snap back, this membrane acted, as one would expect, much like a rubber band. Before the dissection it was noted that the trachea and bronchi, when pulled upon, showed the usual smart recoil. After the elastic membrane had been removed, however, the recoil was very sluggish indeed, and might hardly be said to exist. This demonstrated quite conclusively that the elastic membrane is by far the most important element in the recoil mechanism of the respiratory tract.

#### STAINED AND CLEARED WHOLE MOUNTS

The forked elastic strap, obtained as above described, was wrapped loosely around a small shell vial, tied, and fixed in 10 per cent neutral formalin for twenty four hours. It was then washed, dehydrated, and stained in toto in Weigert's elastic tissue stain. Then, after alcoholic extraction and clearing in benzine and oil of wintergreen, the specimen was studied by the binocular and compound microscopes.

The elastic membrane as thus prepared was found to be much too dense for the study of the individual elastic strands, which, to be made visible, had to be spread apart somewhat with the aid of dissecting needles. Especially was this true when the specimen was to be examined with the compound microscope. Often a slender piece had to be dissected off and teased carefully with needles in oil of wintergreen before the individual fibers could be plainly seen, so numerous and closely approximated were they.

Examinations of preparations made in this way disclosed the following facts regarding the elastic fibers. They run, as the authors have pointed out, in a longitudinal direction, i.e., parallel to the axis of the tube in the wall of which they appear. Each fiber follows a gently undulating course, and they branch frequently, so that there is formed in this way a meshwork of elastic fibers, the meshes being elongated longitudinally. The fibers are of different sizes, the average being much larger than that of those found immediately beneath the epithelial layer. The



largest fibers are quite equal to, if not greater than, the largest of the adventitia fibers. Their branching is after the manner typical of elastic fibers, there being a sharp angle where the branch is given off. The meshwork which they form is very close and dense. Where branching of the bronchus occurs, the membrane may be followed uninterruptedly into the diverging limbs, and, while upon the saddle-shaped ridge at the apex of the acute angle formed by the bronchial branches the fibers are not so characteristically longitudinal, yet they take this form immediately below the fork. There is a tendency for the fibers to be arranged in fasciculi, which themselves interbranch. In the trachea the fasciculi are flattened and the branching is more frequent, whereas in the bronchi, especially in the larger ones, the fasciculi are stouter and run for considerable distances without branching. Here, too, the entire membrane is especially dense, the fasciculi being very conspicuous.

Thus the elastic membrane forms a complete extensible sheath for the epithelium of the respiratory tract. If we were to dissolve away all but the elastic membrane from the bronchial tree, its form would still be perfectly represented. It is a veritable *arbor elastica*.

Small strips similarly prepared from the respiratory tract of the sheep and dog show the same general condition.

#### APPEARANCE IN CROSS-SECTION

Illustrations of cross-sections of the elastic membrane of the respiratory tract and descriptions based on examinations of cross-sections are to be found in the literature, and I shall here give only a brief account of a typical specimen. A fresh sheep's lung, together with the trachea, was obtained and fixed in formalin. The lung tissue was then dissected away, exposing the bronchial tree, which was photographed. The specimen was then washed, dehydrated, and stained en masse with Weigert's elastic tissue stain. After extraction and clearing with benzine and oil of wintergreen, pieces of trachea and left main bronchus were cut out, carefully numbered, and embedded in paraffin, the situations of the blocks thus obtained being marked upon the photograph of

the entire specimen. Thus the exact location of each section is known. Sections were cut from these blocks and either mounted as they were or counterstained. In this way sections were obtained of representative regions of the entire airway.

The general appearance of the elastic membrane, in cross-section, is much the same throughout the entire respiratory tract. The low power shows a sharply marked dark blue ring in the deeper layer of the tunica propria, which is resolved, by the higher powers, into a ring of thickly set blue dots—the cut ends of the elastic fibers. This ring of fibers is very conspicuous indeed in these preparations. By focusing up and down, branchings can be made out. The fiber-ends are of different sizes and often they occur in close groups; these groups, together with single strands, being collected into fasciculi, as seen in the whole mounts. In the larger bronchi this grouping and fasciculus formation is especially conspicuous and the fibers are very large. The longitudinal folds present in the mucosa of the bronchi in some animals were practically non-existent in my specimen of sheep's bronchus.

From the main ring of longitudinally disposed fibers, in the larger tubes, a few small elastic fibers can be seen to spring medially, and to be disposed in a loose, plexiform manner immediately beneath the epithelium, in the thin inner layer of the tunica propria. These fibers are much finer than the average size of those of the elastic membrane, and run in various directions, many of them circularly. Luciani ('11) and others mention them. They seem to serve the purpose of maintaining an elastic contact between the elastic membrane and the epithelial layer so that the latter will quickly and easily follow the movements of the former. A certain amount of freedom of the epithelial layer upon the elastic membrane is allowed.

Laterally, too, fibers are given off from the elastic membrane which pass out to mingle with the fibers of the adventitia. Some are connected loosely with the elastic tissue of the perichondrium surrounding the cartilages and others are interwoven with the muscle fibers. These adventitia elastic fibers form a loose felt-work, the strands running in various directions, but the impression is that most of them follow the direction of the bronchi with which they

are associated. They are not closely packed, like the fibers of the elastic membrane, nor do they make up, collectively, nearly such a mass of elastic material. Thus their action in shortening the respiratory tract, in the bronchi at least, must be very meager compared with that of the elastic membrane. In the lower end of the trachea, however, they were quite abundant. Connection of the fibers of the elastic membrane with the cartilages, muscle, and connective tissue of the outer layers, although loose and slight, yet probably makes for a smooth and orderly shortening of all layers of the respiratory tract during the contraction of the elastic layer.

As the elastic tube is followed down the bronchial tree, it is seen to diminish in density with its reduction in cross-section, and the fibers become of smaller average size, but even when the smallest bronchi are reached it is still a distinct layer. Indeed, it is here quite conspicuous, its main features—position, longitudinal direction, and closeness of apposition of fibers, etc.—being the same as in the larger bronchi. In the bronchioles the elastic membrane forms a conspicuous sheet underlying the muscle. Here, too, a large number of fibers lie outside of the muscle layer, and many of them run longitudinally; these fibers are often connected with the elastic membrane. As the bronchiole is followed down, the elastic membrane is seen to become gradually thinner and its terminations are in connection with the elastic tissue of the walls of the terminal air chambers, as many authors point out (Foster, '93, p. 436, et al.).

#### DISCUSSION

From a study of the structure of the bronchial tree, two facts emerge with clearness: that this branched system of tubes is *extensible*, and that it is *capable of recoiling* to its original condition.

That it is extensible is plainly deducible because longitudinal elastic fibers are such a prominent part of it, and because the tissues holding together the cartilages are of a loose character and are capable of being stretched to some extent. More convincing still is the experimental evidence; we are able to stretch the isolated tubes of the system in a fresh preparation. Every

branch of the tree is capable of being drawn out, and when they are all stretched the volume enclosed by the boundary marked out by their terminal points is much greater than that enclosed by these points when the branches have contracted. This, it is plain, is a phenomenon inextricably bound up with the expansion of the lung; in fact, it is one of the most outstanding features of this expansion.

In expansion of the lung, then, the bronchi stretch. In incomplete inspiration, of course, they may not all do so; some portions of the lung, particularly in the upper lobes, as Keith ('09) has pointed out, may be unexpanded in ordinary quiet breathing, and in individuals leading a sedentary life these regions may be practically unused. But in the healthy lung, in full inspiration, all the limbs of the bronchial tree are stretched, and, as we shall see, the trunk of the tree—the trachea—is also stretched.

If a demonstration were needed to convince us that there must be a stretching of the bronchial tree in inspiration, it would be afforded by a comparison of the levels marked out by the lower margin of the human lung in full inspiration and in full expiration. As every clinician knows, there is an interval of considerable magnitude here. Writing on the human lung, Norris and Landis ('17, p. 101) state that the "respiratory displacement of the lung is most marked in the axillary line" and that "during forced breathing the excursion may amount to 9 cm. ( $3\frac{1}{2}$  inches)." The amount of the descent of the diaphragm, particularly in forced breathing, is evidence of the same kind. Starling ('20, p. 1091) states that this excursion, at the domes, in quiet breathing averages about half an inch, and Norris and Landis ('17, p. 101) give the corresponding figure as three-quarters of an inch. The latter authors state that in forced respiration the interval may amount to  $2\frac{1}{2}$  inches, and even as much as 5 inches. Such facts can mean only that the lung is stretched along its sagittal axis during inspiration, since there is but slight, if any, downward movement of the apical region during the inspiratory phase.<sup>1</sup> Some writers consider

<sup>1</sup> Keith ('09, p. 187) says: "During inspiration the apical resonance does not extend into the neck, but decreases (Colbeck), and by placing a tambuur on the neck over the apex of the lung it is found that the apex descends whenever the diaphragm is well in action, even if the first rib remains stationary."

that there is even at the apex some slight inspiratory expansion upward (Norris and Landis, '17, p. 97) as well as outward and forward. Thus the piston-like action of the diaphragm stretches the entire lung upon its long axis. The lung is stretched also, of course, anteriorly and laterally. The useful diagrams of Keith (Hutchison, '09, pp. 196-198) show graphically all these movements. Such a marked stretching of the lung substance can only occur with a stretching of the bronchial tree, for such a result would be impossible if it were inextensible. Hutchison ('09, p. 180) believes that the trachea and large bronchi stretch during inspiration.

In this connection Miller's ('21) results are interesting. This investigator made casts of the main bronchi and branches of the lower right lobes of the lungs of two dogs of equal size and weight, one lung being in the condition of full expansion and the other of total collapse, and found upon comparing them that both the main stem bronchus and the branches were longer in the expanded lung. While not positive proof of extension of the tubes, since the comparison could not be made of the collapsed and filled conditions in the same lung, and since a totally collapsed lung was used (instead of one in the condition of moderate distension normal for the expiratory phase) for comparison with the expanded one, yet the results go far to convince one of an elongation of the bronchial tree in inspiration. It may be remarked here, too, that Miller's (21) studies upon the muscle bands in the bronchi and bronchioli lead him to conclude that (p. 703) "When the action of these bands of muscle is studied it will be found that the arrangement is such that provision is made for the elongation of the bronchi and bronchioli during inspiration and the subsequent shortening during expiration."

It is evident, then, that the bronchi and bronchioli are stretched during inspiration, and particularly during forced inspiration. But there is, also in forced inspiration at least, a stretching of the trachea. Keith ('09) has emphasized the fact that the root of the lung, together with the heart, undergoes a descent with inspiration accompanying the descent of the diaphragm, and Macleod ('20), following Keith, has this to say (p. 343): "The

root of the lung, which has generally been regarded as more or less fixed, undergoes in normal breathing a definite forward, downward and outward movement, and the heart shares in this movement (Keith).” Others have noted the same phenomenon, vide Groedel’s (’13) diagram on page 206 of Norris and Landis (17). X-ray study of the diaphragm movements shows that the central portion has an up-and-down excursion, and Macleod (’20, p. 338) states that “The central portion of the diaphragm does not move much in normal respiration, but in forced respiration its movements may be considerable.” Thus the lower end of the trachea descends with deep inspiration, and the tube is stretched. The stretching of the entire airway in full inspiration is in keeping with the essential unity of its system of elastic fibers, and particularly those of the elastic membrane. The fact that the bronchus enters the hilus of the lung very obliquely favors its free movement here.

To realize the importance of the stretching of the bronchial tree, it is only necessary to imagine it to be perfectly inextensible. It seems obvious that then the free expansion of the lung tissue would be impossible.

The stretching of the bronchial tree bears some resemblance to the stretching of a rubber band where one end is fixed and the other pulled upon. The movement of different points upon the band becomes greater and greater as we progress from the fixed end to the end pulled upon; so, too, the movements of points situated at different levels of the respiratory tract are least in the region of the larynx and greatest at the termination of the system.

From what has already been said, it is apparent that the free expansion of the upper end of the lung is largely dependent upon the downward movement of the lower end, for the upper portion cannot expand downward until the lower part has made way for it. The upwardly directed bronchial radicles must have room to elongate. True, the upper end can expand outward and forward, and there may be, as we have said, some slight upward expansion, but its filling would be greatly impaired if the downward movement should be impeded. Since this downward stretching of the lung is dependent largely upon the flexibility

of the root region, it follows that anything which interferes with the freedom of movement here will interfere with the free filling of the lung, and particularly with the upper end of it. Is it not possible that the fibrotic changes following inflammatory conditions might cause such an inhibition of normal movement in the lung roots? In this connection Keith ('09, p. 189) remarks as follows: "In cases where the roots of the lungs are bound to the posterior or stationary wall of the thorax through adhesions set up by mediastinitis, Wennekebach observed that both the respiratory and circulatory movements were abnormal in character." We may well ask what would be the results due to the tissue changes consequent upon chronic tuberculous lymphadenitis in the tracheal and bronchial lymph nodes. If such changes tended to fix the roots of the lungs, or to interfere seriously with the extensibility of the bronchi, grave impairment to lung expansion would result, and this impairment would be particularly in the upper lobe. Is it not possible that the greater frequency of tuberculosis in the upper lobes of the lungs than in the lower is due to the crippling of the expansion in the upper lobe consequent upon inhibition or elimination of the normal movements of extension and contraction in the trachea and main bronchi? One cannot conceive how a stiffening of any important part of the bronchial tree could be without its damaging effects upon the function of the area of lung supplied by it.

#### THE RECOIL MECHANISM

It is apparent to anyone who makes a careful study of the elastic membrane of the trachea and bronchi that we have in it to deal with the densest aggregation of elastic fibers in the whole respiratory system below the larynx. In comparison with this membrane, the density of the remaining elastic tissue of the airway and that surrounding the terminal respiratory chambers is relatively slight. This, together with the further facts that the fibers run parallel with the various limbs of the bronchial tree, as well as with its trunk, and that they are attached above to the relatively fixed larynx, forces upon us the conclusion that we have here to do with a very important, if not the main, element in the

elastic recoil mechanism of the lung. It is quite obvious, too, that to insure an efficient retraction of the entire lung substance, this retraction should be centered along the axes of the lung substance, viz., along the bronchi. Immediately upon the relaxation of the inspiratory musculature of the thorax there is a recoil of the elastic tissue of the entire lung substance. There is a shortening of the entire bronchial tree, together with a diminution in the space within the respiratory tissue proper. This even shortening of the bronchi prevents bending or buckling of the tubes, with resulting partial or complete obliteration of the lumen, which would occur if the lung tissue should recoil without a coincident retraction of the limbs of the bronchial tree. It is by a combination of the recoil of the respiratory portion of the lung, of the pleura, and of the airway proper that the air is driven out in a smooth and rapid manner, without the trapping of air due to wrinkles or folds occurring in the bronchi. The entire system of elastic tissue works together, and the action of one part cannot be considered without coordinating it with that of the remainder.

The situation of this elastic membrane—in the tunica propria close to the epithelium—is most favorable for the insuring of a smooth mucosa. Luciani ('11, p. 404), Foster ('93, p. 435), and others mention this function of the elastic membrane, particularly for the trachea.<sup>2</sup> The loose connection of the membrane with the epithelium through the finer network of fibers in the vascular and adenoid layer enables the epithelial layer to follow the movements of the membrane without being torn or wrinkled. Were it placed between the cartilages of the airway, its total length would be enormously reduced, and its efficiency correspondingly decreased; then, too, transverse folding of the mucosa, and perhaps tearing of it, would be apt to occur. Still more unfavorable, perhaps, would be a situation farther out—beyond the cartilage zone—for then would occur an inevitable partial or

<sup>2</sup> The continuity of the fibers of the elastic membrane above with the abundant elastic tissue of the larynx permits of stretching of the trachea in upward movements of the larynx, as in swallowing or bending the head backward, and of returning to the normal position (Hutchinson, '09, et al.).



complete obliteration of the lumen, not only due to the forcing into it of the cartilages of the walls, with the contraction of the elastic fibers, but also on account of transverse folding of the epithelial layer. Situated as it is, the mucous membrane is able to follow the extension and contraction of the airway without injury and without offering any impediment to the airflow.

#### BLOOD VESSELS AND OTHER STRUCTURES

It seems apparent that the blood vessels which accompany the bronchi should be capable of stretching, or at least of straightening out, and of returning to the unextended condition, to follow the movements of the bronchial tree. It is not unlikely, too, that they should show some difference in respect to the arrangement of their elastic tissue in comparison with other vessels of the body which are not forced to undergo like deformation, and this point is being investigated. It is doubtful, however, if we have in the vessel walls any appreciable part of the recoil mechanism of the lung. Similarly, the structure of the lymphatics and nerves must permit of their being stretched.

#### OSCILLATORY MOVEMENTS OF THE BRONCHI?

Keith ('09), Miller ('21), and others have favored the view of a widening and narrowing movement in respiration, which is said to occur in the angles or spaces between diverging bronchial branches. In inspiration the branches which center at a common point separate from one another, like (as Keith puts it, p. 184) "the opening of a Japanese fan." This conception seems to rest upon the idea that the lung tissue enclosed by two or more convergent bronchial limbs expands, thus forcing them apart, and so the available space is increased.

There are some difficulties in the way of too liberal an acceptance of this notion. The bronchial tree, for instance, is not built quite like a Japanese fan. In opening the fan the lateral ribs must swing through a very wide arc, and such an extensive swaying movement would be impossible for the bronchi. Moreover, the idea fails to take into account the fact that these bronchi which are supposed so to move apart are surrounded on all sides by

expanding lung tissue (assuming expansion to be normal and full), and that the tendency toward widening of the angles caused by the expansion of the tissue between the limbs would be largely, if not altogether, neutralized by the expansion of the tissue on the opposite sides of the limbs. Such a movement, if it did occur, might widen the bronchial tree, but could not lengthen it, and so would not account for the downward stretching of the lung in inspiration.

Miller's ('21) efforts in search of the truth in this connection are open to criticism. He describes and shows us figures of two casts of the bronchial trees from the lower right lobes of the lungs of two dogs of equal size and weight. Both specimens, however, were made from lungs removed from the pleural cavity, and accordingly cannot be relied upon as picturing exactly normal conditions. These casts Miller seems to look upon as representing the contrasted forms of the bronchial tree in expiration and in inspiration. Upon comparing them, it is obvious that in the cast said to show the expiratory phase there has been an approximation of the bronchial radicles, but serious objection may be raised against this cast, since it was made from a lung in a condition of total collapse—a condition which is hardly identical with the phase of expiration. The cast purporting to exhibit the phase of inspiration, too, is open to objection, since the lung from which it was made was expanded under artificial conditions. We do not know whether or not the form taken by this bronchial tree is the same as that which would have been taken by it in its normal environment, in the phase of full inspiration. Thus Miller's demonstration does not prove the point which he claims for it.

It behooves us to keep an open mind regarding this question, however, lacking convincing demonstration in favor of either side, and when one looks at a cast of the bronchial tree and realizes that the bronchial limbs point in general toward the regions of greatest expansile movement, one is inclined to admit that there is something in the idea of bronchial inspiratory divergence. However, with the idea before us of an extensible bronchial tree, it is not necessary to place so much dependence upon this Japanese-fan concept to explain lung expansion, and, in any event, such a

movement under existing conditions, would be absolutely inadequate to account for lung expansion and contraction without having associated with it an extension of the limbs of the bronchial tree.

One should note, too, with Keith ('09) that the expansile movements of the lung in the transverse plane are practically limited to the outward and forward (and intervening) directions, there being no opportunity for the lung to expand backward, and but little or none inward. Even when we allow for the in-drawing of the mediastinal walls, due to the elongation of their contents, which follows the descent of the central part of the diaphragm (Keith, '09) in vigorous inspiration, it is plain that the main directions of expansion of the lung, in the transverse plane, are as Keith has pointed out. It follows, then, that if the tissue lying between the main bronchial stem and the posterior and medial surfaces is to expand, it must be by the shifting outward of this main bronchial stem. This would mean some slight divergence of the two main bronchial stems from one another.

#### SUMMARY

1. The conspicuous network of longitudinal elastic fibers which forms a membrane in the tunica propria of the entire bronchial tree of mammals, and which I have referred to as the "elastic membrane of the bronchial tree," may be dissected off en masse from the fresh trachea and larger bronchi, and the branched strap so formed behaves when stretched much like an elastic band.

2. The specimens so obtained may be stained, cleared and mounted for study.

3. This membrane, when considered as an isolated unit, is in general form a replica of the entire bronchial tree, and its density corresponds fairly directly with the size of the tube in the wall of which it is found. It becomes thin and delicate in structure toward the ends of the bronchioles, and is connected with the elastic tissue of the respiratory portion of the lung. It constitutes a unified system of branched elastic tubes which extends from the larynx to the outermost limits of the air channels.

4. Each of these tubes represents an axis for the extension and contraction of the lung substance, and in the expansion

and recoil of this substance the movements follow these axial lines. The recoil of the lung substance, considered collectively, is thus a summation of the recoil of its various elements, centered along these bronchial axes. The structural unity of the system emphasizes its functional unity.

5. Evidence is reviewed to show that the bronchial tree stretches in inspiration and contracts in expiration. The actual excursion of any point within the bronchial tree is greatest at the extremities of the limbs and least at the upper end of the trachea. The tips of the caudally directed twigs of the lower lobe show the greatest excursion. The amount of stretching, of course, varies with the depth of the inspiration.

6. The filling of an area of lung tissue with air is associated with a stretching of the air-carrying tubes supplying it. With a rigid bronchial tree the movements of expansion and contraction of the lung, as we know them, would be impossible.

7. The elastic membrane is by far the densest aggregation of elastic fibers in the entire bronchial tree, and this fact, together with the consideration that its fibers run parallel with the axes of the various branches of the tree, and that the bronchial tree, deprived of this membrane, suffers a loss of most of its ability to recoil, forces us to the conclusion that in this membrane we have by far the most efficient part of the recoil mechanism of the entire bronchial tree. When, further, the relative amount of the elastic tissue in this membrane is compared with that in the remainder of the lung substance, we realize that we have in this system of elastic tubes a very important, if not the most important, portion of the recoil mechanism of the entire lung.

8. The conception of a stretching and contracting of the bronchial tree in respiration minimizes the importance of the conception of an oscillatory movement on the part of the bronchial branches, after the manner of the ribs of a fan. It seems quite possible that the main bronchial stems may shift forward and outward in inspiration, thus providing for the expansion of the vertebral and mediastinal region of the lung tissue.

9. Much of the expansion of the upper lobe is secondary to the downward shifting of the entire lung, and it follows that

freedom of movement in the lung root is essential to freedom of movement of the lung, particularly in the upper lobe. Thus, pathological conditions which tend to immobilize the roots of the lungs (such as tuberculosis of the neighboring lymph glands and associated adhesions) will tend to cripple the movements in the upper lobes, and so may help to account for the selective affinity of this region of the lung substance for the tuberculous process. More widely, anything which acts to stiffen the limbs of the bronchial tree will affect adversely the function of the part supplied by these limbs, and so will tend to precipitate pathological processes therein.

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Abstracted by Eliot R. Clark and Eleanor Linton Clark, authors.  
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The reaction of living cells in the tadpole's tail toward  
starch, agar-agar, gelatin, and gum arabic.

Small droplets of corn- or arrowroot starch were injected into the transparent tails of frog larvae. Uncooked, semicooked, and completely cooked starch were studied. The results were studied in the living animal, and also after staining with iodine. Uncooked starch causes a mild attraction for leucocytes, which phagocytize the granules and retain them unchanged as cell inclusions indefinitely. Boiled starch exerts a moderate attraction for leucocytes; it is quickly transformed by substances both outside and inside the leucocytes into colorless dextrin or sugar. Semicooked starch granules exert a most intense attraction on leucocytes, which surround the granules, phagocytize the smaller ones, and, in the course of a few hours, transform them into substances which do not stain with iodine. Agar-agar, gelatin, and gum arabic, introduced in capillary glass tubes, exert an attraction on leucocytes similar in kind and intensity to that exerted by semicooked starch. The leucocytes approach and phagocytize them rapidly. The other tissue cells show no response toward starch or the other substances used.

## THE REACTION OF LIVING CELLS IN THE TADPOLE'S TAIL TOWARD STARCH, AGAR-AGAR, GELATIN, AND GUM ARABIC

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### SIX FIGURES

During the past twelve years the authors have studied the character, mode of growth, and behavior of living cells in the transparent fin expansion of the tails of amphibian larvae. By means of chloretone anesthesia and an upright glass chamber, or microaquarium (Clark, '12), it has been possible to see individual cells and tissues with great clearness and in their normal location, to follow the same cells for hours, days, and even weeks, and to watch their growth and their response to various stimuli. The cell types especially studied have been connective-tissue cells, wandering cells, and blood-vessel and lymphatic endothelium. The response of these cells toward a number of injected substances has been described in previous communications.

The success of the injection experiments is largely dependent upon the use of substances which are readily injectable through fine glass cannulae, thus producing a minimum of injury, which will remain unabsorbed long enough to allow time enough for an observable reaction on the part of the cells and tissues, and which will be visible. Suspensions of granules, such as carmine and carbon, fulfill these conditions and have been used in experiments of this kind. Oily substances also lend themselves to such experiments and their effect has also been studied. Small globules of an inert oil (paraffin oil), nutritive oils (olive-oil globules and emulsions of yolk of egg and cream), and an injurious oil (croton oil) are the substances of this category which have been used in previous experiments.

Substances which may be dissolved or suspended in paraffin oil can be used for such experiments, since former observations showed that paraffin-oil globules are inert when inserted into the tails of tadpoles. With other more easily diffusible substances it is possible to fill a small glass cannula (measuring 10 to 20  $\mu$  at the tip) with the substance to be tested, to insert it in the fin in the desired position, and then to break off the end, leaving a small glass tube open at both ends inside the fin. The epidermis heals over the opening within a few minutes. This method is not satisfactory, however, unless the substance contained in the tube is either stained or mixed with some visible material.

In the first series of experiments reported here, starch, in small enough quantities to permit of distinguishing individual granules, was injected through fine glass cannulae into the transparent tail fins of living tadpoles. Later, the effect of other substances of somewhat similar physical constitution, such as agar-agar, gum arabic, gelatin, etc., was studied.

#### EXPERIMENTS WITH PLAIN GLASS TUBES

Since it was found impracticable to inject uncooked starch grains, agar, celloidin, gum arabic, and gelatin on account of the great difficulty encountered in trying to force these substances out of the tips of fine cannulae, these materials were drawn up into small glass needles which were then inserted in the fin, the end broken off, and the tube tucked under the skin in the desired position. The glass tubes then served as small test-tubes, open at both ends, through which the enclosed substance could slowly diffuse into the surrounding tissue.

Control experiments were performed with plain glass tubes—empty except for the surrounding medium of sterile chloretone solution—which were inserted in the manner described above. By a study of the reaction produced by these tubes it was possible to distinguish between the response of the living cells to the various substances which we wished to test and the reaction to the mere presence of a foreign body.

The presence of such a glass tube served as a stimulus for a rather severe epidermal reaction which took place soon after its



insertion. The epidermal cells became opaque and pigmented and had the appearance of being heaped up on that part of the surface immediately over the ends of the tube. This reaction of the epidermal cells was more marked when longer tubes were used than in the case of the very short ones. It subsided during the first twenty-four hours after the operation.

Near-by wandering cells approached the tube and flattened out on its surface and, an hour or more after the insertion of such an empty glass tube, a slight stickiness of leucocytes to the wall of a few blood vessels in the vicinity was observed. A few leucocytes were seen to migrate from these vessels toward the tube. During the next two days the leucocytes and wandering cells lined up along the tube, forming a single layer of flattened cells which completely surrounded it. After the formation of such a cellular layer, the supernumerary leucocytes wandered away from the region. Such tubes were observed for periods of two to four weeks, and, after the formation of this leucocytic layer, they apparently exerted no influence on the surrounding cells and tissues.

The insertion of plain glass tubes into the tadpole's tail evidently produces a pronounced but transitory reaction of the epidermal cells of the region. Their presence also attracts leucocytes from near-by blood vessels to a slight degree. In other words, these tubes produce a mild form of foreign-body reaction.

#### THE REACTION OF LIVING CELLS TO STARCH GRANULES

Corn-starch and arrowroot were the two kinds of starch used. They were injected in three different forms: 1) as uncooked starch, 2) as cooked starch, and, 3) as semicooked starch (cooked just to the point of gelatinization). The uncooked starch was treated with ether, which was allowed to evaporate, and the starch then suspended in sterile distilled water. The cooked starch was prepared by mixing starch with distilled water and boiling for ten to fifteen minutes. 'Semicooked' starch was made by mixing the starch with sterile distilled water and heating in a water-bath to the point of gelatinization.

In the case of the two varieties of cooked starch, it was possible to inject small quantities of the paste into the tail fins by means of the small glass cannulae. With the uncooked starch, however, the individual grains are too resistant to permit of their being forced through the small cannulae, consequently small glass tubes containing uncooked starch grains were inserted under the skin in the manner just described.

The experiments were performed on larvae of *Rana pipiens*, *Rana catesbiana*, and *Bufo lentiginosus* Fowleri.

Chloretone anesthesia and the same methods of injection and observation described in previous articles were employed (E. R. Clark, '12, '16).

After observing the cells in the vicinity of the injected starch for varying intervals of time, the tadpoles were fixed in tincture of iodine (in 70 per cent alcohol). The tails were then cut off and cleared in graded strengths of glycerin.

The microscopic appearance of the three forms of starch is different. The uncooked starch grains are round refractile bodies with sharp, regular outlines. The grains of cooked starch are translucent and irregular in shape. The semicooked granules are opaque and rounded. In the preparation of both cooked and semicooked starch, many of the granules swell and burst, discharging their contents into the surrounding medium, leaving only the shells of the former granule.

#### I. EXPERIMENTS WITH UNCOOKED STARCH

In a number of tadpoles, a glass tube containing uncooked starch was inserted in one fin and a similar tube containing cooked starch in the other. Wandering cells from the tissues and leucocytes from the blood vessels were attracted toward both tubes, but they migrated in much greater numbers toward the tube containing the cooked starch.

On the morning following the experiment, many leucocytes were present around both glass tubes, but those around the tube of cooked starch were much more numerous. Leucocytes were packed in the lumen of the latter tube throughout its extent, and all of the starch was apparently inside of leucocytes. A few

leucocytes were also present inside the tube of uncooked starch, but the greater part of the lumen was still filled with the unchanged refractile starch grains.

On the second day after the insertion of the tubes the central portion of the tube of cooked starch was empty, while leucocytes, granular in appearance, were still numerous around the ends of the tube. The tube of uncooked starch still contained many free granules. A number of leucocytes containing refractile starch grains were found at the ends of the tube, in the surrounding tissue spaces, and inside of near-by lymph vessels.

The glass tubes were usually extruded from the tail after a week or ten days. In the case of the tubes of uncooked starch, a number of leucocytes containing refractile starch grains were left behind in the tail fin. During the subsequent days many of these leucocytes wandered away from the injection site, some of them entering lymph vessels. However, a number still remained near the original site of the tube, and these were followed for several days and, in one specimen, for as long as a month with no change in the appearance of the starch grains inside of leucocytes. Uncooked starch grains, therefore, acted apparently like foreign bodies, such as minute drops of paraffin oil or particles of carmine and carbon. Wandering cells and a number of leucocytes from the blood vessels were attracted toward the uncooked starch and they proceeded to ingest the starch grains, which then remained as cell inclusions for practically indefinite periods of time. It is probable that the cellulose covering of the uncooked granules prevents action on the granule by the ferments in the leucocytes and tissue fluid.

## II. EXPERIMENTS WITH COOKED STARCH

When boiled starch paste was injected into the tail fins of tadpoles, the starch granules appeared as translucent bodies with irregular pliable-appearing outlines. These starch grains disintegrated rapidly and disappeared after fifteen to forty-five minutes (depending upon the quantity injected). In one case only three granules of cooked arrowroot were injected in one region. These three were watched carefully. At ten minutes after

the injection, all three granules were mere shadowy outlines and sixteen minutes after the injection they had disappeared altogether. The tadpole was immersed immediately in a weak solution of iodine and then cleared in glycerine. Microscopic examination disclosed a faint bluish tinge at the site of injection. In this case the starch grains had dissolved without any visible reaction on the part of the surrounding tissue cells and before any wandering cells or leucocytes had reached them.

In other cases, in which a larger quantity of cooked starch was injected, some grains were still present a half-hour after injection and leucocytes and wandering cells moved toward the injected starch and engulfed the undissolved portions of the starch grains. Such a specimen when treated with iodine one-half hour after injection showed two large blue granules with leucocytes adherent to them, a scattering of finely granular almost diffuse blue stain, and several leucocytes with a bluish tinge in their cytoplasm. Tadpoles, fixed in iodine, one hour or more after injection of thoroughly cooked starch, showed no trace of blue color.

These experiments, as well as those just described in which the starch was introduced into the fin inside of glass tubes, showed that cooked starch is a stronger chemotactic stimulant for leucocytes than uncooked starch. They also showed that the tissue fluid itself possesses some substance capable of dissolving and digesting cooked starch grains.

No cells except leucocytes were affected in any visible way by the presence of the starch.

### III. EXPERIMENTS WITH SEMICOOKED STARCH

Starch (in the form of corn-starch or arrowroot) was mixed with sterile distilled water and heated over a water-bath just to the point of gelatinization. In this case the starch grains are pliable enough to permit of injection through a fine glass cannula, yet substantial enough to remain as discrete rounded bodies which do not dissolve in the tissue fluid. These semi-cooked starch grains are rather opaque and easily distinguishable from the round refractile uncooked starch grains, as well as

from the translucent, irregularly shaped grains of cooked starch. Starch grains prepared in this way were injected into the tail fins, the region studied, and camera-lucida tracings made of the region near the injection.

The near-by connective-tissue cells, blood vessels, and lymphatics showed no reaction to the presence of the starch. Wandering cells, located near the injection site, showed an immediate response: they made their way directly toward the starch and their movement was very rapid.

Ten to fifteen minutes after injection, leucocytes were observed migrating from near-by blood vessels. In contrast to the experiments with croton oil, the leucocytes in this case came out of the vessels situated nearest the injected material, instead of those further away. These leucocytes moved swiftly toward the starch and, arriving at the site of injection, they proceeded to surround the individual starch granules. More leucocytes continued to come out of the blood vessels and to arrive upon the scene until, after a period of one to three hours (the length of time depending upon the amount of starch injected), no starch grains could be seen, the injection site being occupied by a dense mass of leucocytes (figs. 3 and 4).

The diapedesis of leucocytes started sooner than after the injection of any substance tried in our previous experiments; the number of leucocytes which came out of the vessels was very much greater than that attracted toward injected substances, such as powdered carbon or carmine, fats such as olive oil, cream, yolk of egg, or toward the area of inflammation produced by injected globules of croton oil, or by infections. And, too, the movement of the individual leucocytes and wandering cells toward the starch grains was more rapid than that observed in the case of these cells under any other circumstances.

The active migration of leucocytes toward the site of the injected starch continues for several hours after all of the starch has been taken up by cells (fig. 4). This attraction for leucocytes is much more conspicuous in the case of the semicooked starch than in that of either the cooked or the uncooked varieties. In several instances in which cooked starch was injected into

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the fin, a few semicooked granules, easily detected by their more opaque appearance, chanced to be present in the midst of the translucent cooked ones. When the individual starch grains were numbered and watched constantly for several hours, it was observed that the first leucocytes which approached the injected material invariably moved first toward the semicooked starch grains, flattening out on the surface of the grains, frequently passing by cooked grains, and sometimes even moving between two cooked granules on their way to the semicooked ones. In one such case ten starch grains were injected, and of these four were of the opaque semicooked variety. These four were completely surrounded by a mass of migrated leucocytes before any of the cells had approached the more thoroughly cooked granules. In the meantime the cooked granules had begun to dissolve and, in many cases, they were shells or shadows. After the semicooked granules had been completely enveloped by dense masses of leucocytes, the next leucocytes to arrive approached the remnants of the cooked granules and proceeded to ingest them. In other cases an occasional uncooked starch grain, detected by its high degree of refractility, was found in the midst of the injected starch. Such granules were not ingested by leucocytes until all of the cooked starch had been disposed of (fig. 2).

After the leucocytes had taken up the starch, their cytoplasm had a peculiar swollen and granular appearance which distinguished them from the other wandering cells. Some of these leucocytes remained near the site of injection for as long as a week after the injection of the starch.

Fixed specimens of the tails of larvae injected with starch showed that both the polymorphonuclear and the mononuclear varieties of leucocytes were attracted toward the starch grains.

#### THE FATE OF INJECTED STARCH

It has been mentioned already that the uncooked starch remained unchanged for weeks after being taken up by leucocytes. The thoroughly cooked starch starts to dissolve very quickly, as evidenced by the finely granular blue stain visible in the

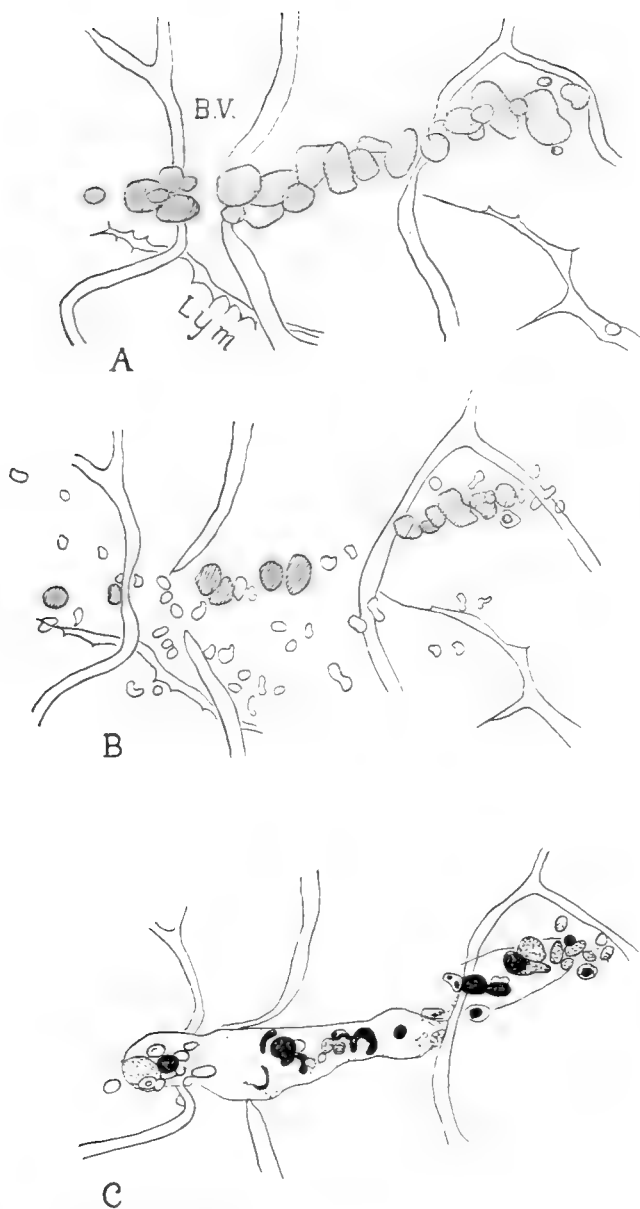


Fig. 1 A. Camera-lucida sketch of the ventral fin of a frog larva immediately after injection of semicooked arrowroot starch. The starch grains are shaded, leucocytes in outline. *Lym.*, lymphatic; *B.V.*, blood vessel.  $\times 105$ .

B. Camera-lucida sketch of the same region one hour later. Leucocytes are migrating toward the starch. Some of the starch has already been taken up by leucocytes.  $\times 105$ .

C. Same, after fixation in iodine. The boundary of the clear space around the injection area is indicated by a line. The portions stained blue with iodine are shown in solid black. The diffuse blue stain with iodine is indicated by dots.  $\times 105$ .

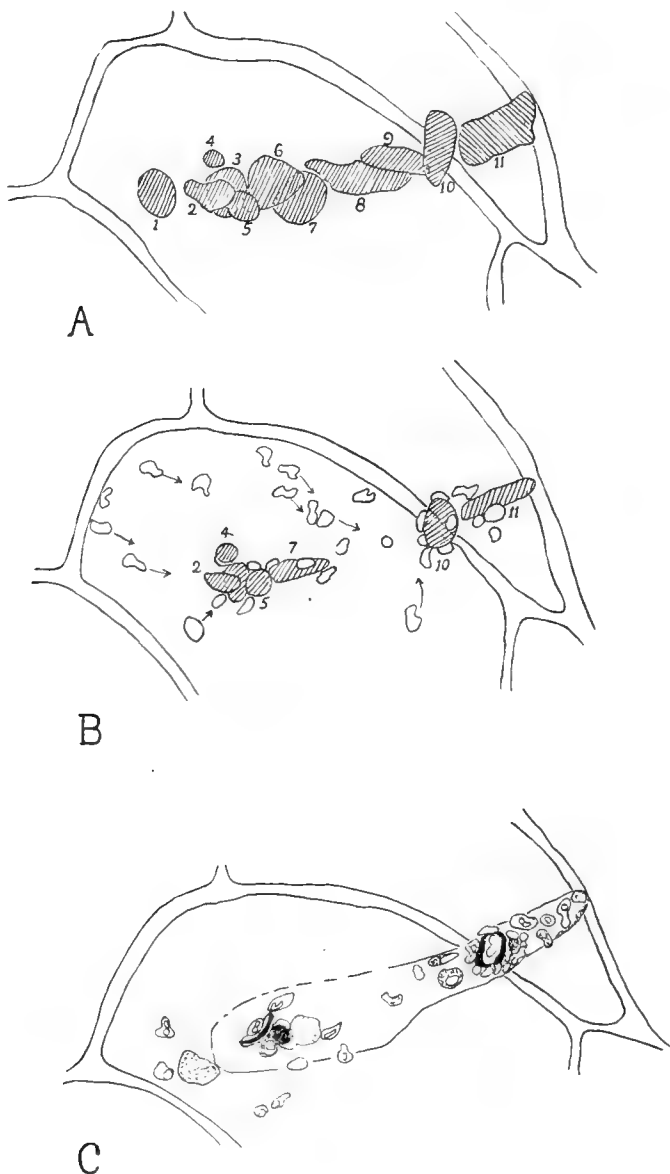


Fig. 2 A. Camera-lucida sketch of ventral fin immediately after injection of arrowroot paste. The starch grains are numbered. No. 4 is uncooked. Nos. 2, 3, 5, 10, and 11 are semicooked. Nos. 6, 8, and 9 are thoroughly cooked.  $\times 123$ .



area of injection, after fixation in iodine at intervals of ten to forty-five minutes after injection.

In the case of the semicooked starch, the starch is apparently converted into a soluble form inside of leucocytes. A series of tadpoles injected with semicooked starch was fixed at different intervals in iodine and then cleared in glycerin. In such a specimen in which only a few starch grains were injected, all of the grains were observed in the living animal to be inside of leucocytes at the end of an hour. After staining with iodine, an hour after injection, a few portions of starch grains showed up as dark bluish-black masses inside of leucocytes. In the cases in which uncooked granules were included in the mass of injected starch these, of course, showed up as blue-black bodies with regular outlines. Still other leucocytes were found with a diffuse blue stain in their cytoplasm. In addition, there were a number of leucocytes collected near the injection site and on their way toward it which were stained a yellowish brown similar to the rest of the cells in the fin. In all specimens fixed an hour to an hour and a half after the injection, a clear space was present which surrounded the area of injection and which contained no connective-tissue cells (figs. 1C and 2C).

In tadpoles, fixed at three hours after the injection of semicooked starch, a number of leucocytes could be found containing a definite blue tinge in their cytoplasm. The blue-black starch grains or parts of grains found in the one-hour specimens

B. Camera-lucida sketch of the same region forty-five minutes after the injection, showing leucocytes migrating toward the starch. The cooked starch grains 6, 8, and 9 have dissolved. Leucocytes have surrounded the semicooked starch grains and others are rapidly approaching them.  $\times 123$ .

C. The same region fixed in iodine one and one-half hours after the injection. Before fixation all of the remaining starch grains had been taken up or surrounded by leucocytes. No. 4, the uncooked starch grain, was inside a leucocyte which had moved it to a position between Nos. 2 and 3. The place of granule No. 10 was occupied by a clump of leucocytes. The iodine fixation shows No. 4 (uncooked) inside a leucocyte, distinct and unchanged in form. Parts of grains Nos. 3 and 10 appear as blue bodies surrounded by leucocytes. The other grains have been dissolved and many of the leucocytes show a bluish tinge in their cytoplasm (black dots in sketch). Some of the leucocytes are definitely polymorphonuclear, others mononuclear. The clear space around the injection area shows in this specimen as in figure 1, C.  $\times 123$ .

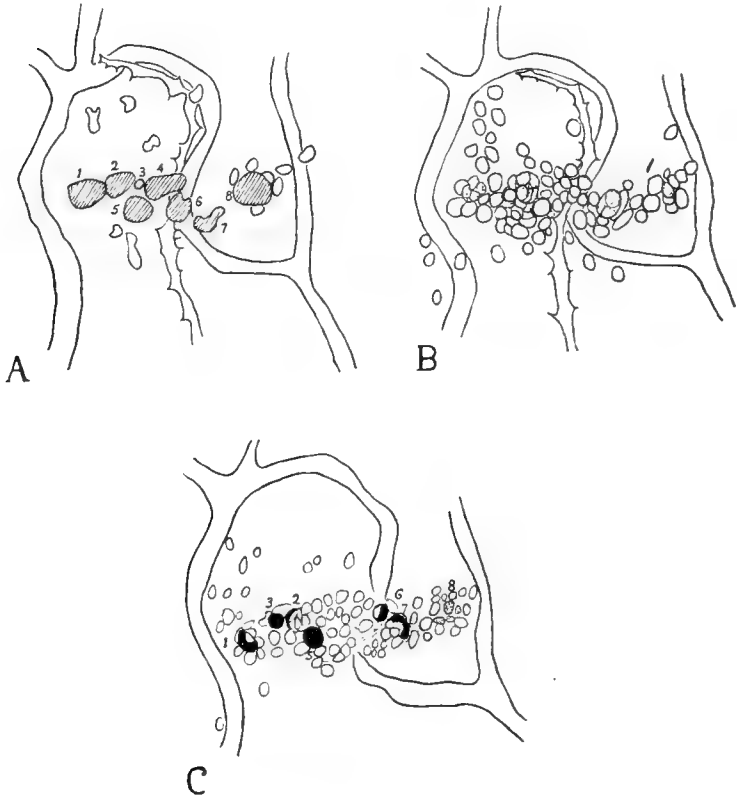
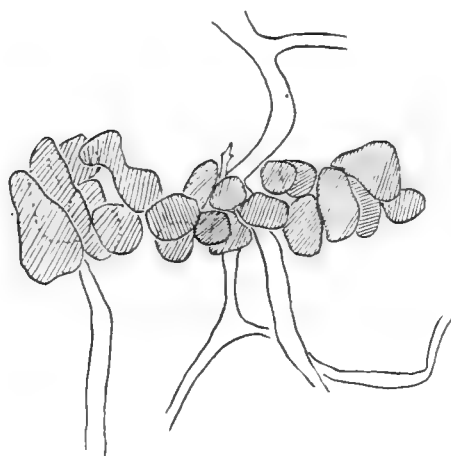


Fig. 3 A. Camera-lucida sketch of ventral fin, twenty-eight minutes after injection of starch (arrowroot). Grains Nos. 3 and 5 were uncooked, the others semicooked. A number of leucocytes have already reached granule No. 8, the others are approaching the injection site.  $\times 105$ .

B. The same region three hours after the injection. Numerous leucocytes present in the injection area and others migrating toward the former site of the starch. The starch grains have all been taken up by leucocytes. Grains Nos. 3 and 5 still show plainly as round refractile bodies. Clumps of granular leucocytes occupy the former sites of the other starch grains.  $\times 105$ .

C. Same region after fixation in iodine. Nos. 3 and 5 show as dark blue bodies unchanged in shape (black in sketch). Parts of Nos. 1, 2, 6, and 7 also stain blue with iodine. Several leucocytes near the former site of No. 8 have a bluish tinge (dots in sketch). No clear area at this stage  $\times 105$ .



A



B

Fig. 4 A. Camera-lucida sketch of area immediately after injection of arrowroot starch.

B. The same region six hours later, showing violence of leucocytic reaction caused by the presence of semicooked starch.  $\times 123$ .

were not seen here except of course in those specimens in which uncooked starch grains had been injected along with the others. The clear space around the injected area was absent in specimens fixed three hours after injection (fig. 3C).

In larvae fixed in iodine at five to six hours after injection of semicooked starch, the blue had entirely disappeared with the same exception of the uncooked starch grains). The mass of leucocytes at the former site of the starch granules contained no trace of blue, but was dark brown. However, when these cells were examined with high magnification the brown color was found to be confined chiefly to the nucleus, the cytoplasm being a pale yellow and there was no noticeable color difference between the cells collected near the injection site and the other wandering cells, while the brown color was not nearly so intense as that found in the muscles.

The iodine reaction showed that the semicooked starch is taken up by leucocytes by which it is dissolved (the diffuse blue stain) and that after three hours it is changed into another substance. Since the characteristic dextrin and glycogen reactions with iodine were not obtained, it is probable that the starch is changed into a colorless dextrin or sugar. Attempts were made to trace by microchemical tests this further process in the digestion of starch, after the blue color with iodine could no longer be seen. The osazone (phenylhydrazine) test for sugar and the test with Benedict's solution (copper sulphate and sodium hydroxide) were tried with specimens in which starch had been injected one to two hours previously, but always with negative results. Control specimens in which a solution of dextrose was injected into the fin also yielded negative results, although the same solution showed the characteristic reactions on a glass slide. We were unsuccessful in our attempts to detect sugar in the tail fin by microchemical means.

Some experiments were performed in order to compare the results obtained in these experiments with those obtained by treating such starch solutions with ptyalin.

Arrowroot paste, cooked to the point of gelatinization, was treated with iodine and examined on a glass slide under the

microscope. The starch grains were all blue-black in color and in addition a bluish fluid with fine blue granules was present between the starch grains.

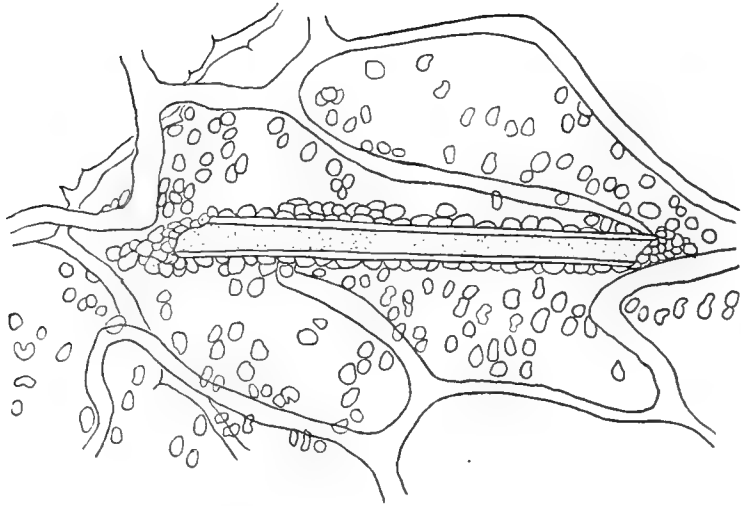
Such starch paste, treated for two to three minutes with ptyalin (saliva) and then fixed in iodine showed some blue-black granules, a greater amount of the finely granular blue material, and, in addition, a few irregular deposits of a pale, yellowish-brown color.

After eight minutes, treatment with iodine showed a number of pale blue, and a still greater number of purple and lavender granules. In addition, more of the large irregularly shaped yellowish-brown masses were present, and also a great many small brown granules, smaller than the original starch grains. After still longer treatment with ptyalin—fifteen to twenty minutes—followed by staining with iodine, no blue staining material remained, and only the large yellow-brown masses and the small brown bodies were present.

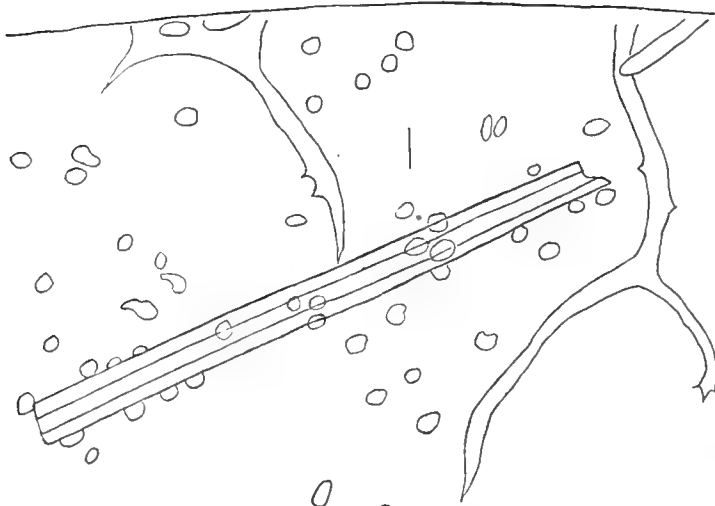
In comparing the fate of starch injected into the subcutaneous region of amphibian larvae with the fate of starch treated with saliva, it will be seen that, in the former case, the starch is changed more slowly than in the latter, and that, after being acted upon, it is apparently changed not into a dextrin or glycogen which stains brown with iodine, but into sugar or dextrin which does not stain with iodine.

#### REACTION OF LIVING CELLS TO TUBES CONTAINING AGAR-AGAR, GELATIN, AND GUM ARABIC

In connection with the reaction of leucocytes to starch granules observations on certain other substances are of interest. These substances were enclosed in minute glass tubes and inserted in the tails of tadpoles by the method described earlier, with the object of discovering a suitable medium for testing easily diffusible substances whereby they might be localized and their too rapid diffusion prevented. Since agar-agar is digested and absorbed to only a slight degree in the alimentary tract, it seemed probable that this would prove a suitable material for this purpose such as paraffin oil proved to be in the case of substances of an oily nature.



5



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Fig. 5 Camera-lucida drawing of the region near a glass tube containing agar-agar, nineteen hours after inserting it in the dorsal fin. Sketch shows large numbers of leucocytes which have surrounded the tube which have collected in great numbers at the two ends, and which are still migrating in large numbers from the near-by vessels.  $\times 123$ .

Fig. 6 Camera-lucida drawing of the region near a glass tube, empty except for a suspension of Berlin-blue granules in distilled water, seventeen hours after inserting it in the dorsal fin. Note the difference in the number of leucocytes attracted toward this glass tube compared with that attracted toward the tube containing agar-agar as shown in figure 5.  $\times 123$ .

The tubes were made, as described, in the form of small glass cannulae measuring 15 to 20  $\mu$  at the tip. The agar was dissolved in boiling distilled water, sucked up into the cannulae while still liquid, and allowed to solidify. A thin layer of agar remained adherent to the outside of the tube. Such a cannula containing agar-agar was inserted in the desired position in the fin, then the tip was broken off and tucked under the skin with the aid of a sharp needle. The hole made in the epidermis healed over within five to ten minutes after the operation. Immediately after the insertion of such a tube the tadpole was transferred to the observation chamber and records made of the region involved.

As might be expected, the epidermal reaction, described in the experiments with the plain glass tubes, took place also in this instance.

Near-by wandering cells moved rapidly toward the tubes of agar. Within ten to twenty minutes after the insertion of the tube, leucocytes began to stick to the endothelium of near-by blood vessels and to migrate through the walls. The leucocytes moved toward the tube and flattened out on its surface, collecting in especially large numbers around the two ends of the tube (fig. 5). Leucocytes continued to adhere to the blood-vessel endothelium, to pass through the walls, and to migrate toward the tube for more than twenty-four hours after the insertion of the tube. After the first two days the migration of leucocytes from the blood vessels ceased, but the leucocytes, both clear and pigmented, still remained flattened against the sides of the tube and heaped around the ends. Three or four days after the beginning of the experiment, the lumen of the tubes was filled with leucocytes which had acquired a granular appearance, apparently as the result of ingesting the agar. Such tubes remained inside the tail for periods of three weeks and over, and at the end of this time the tubes were still filled with the granular leucocytes and many cells were still adherent to the sides of the tube. By this time the rest of the migrated leucocytes had wandered away from the region of the experiment.

Tubes containing substances of somewhat similar constitution were inserted into the tail fins of tadpoles. These were gelatin and gum arabic. They were prepared in the same way as was the agar-agar; that is, they were dissolved in boiling distilled water, sucked into the cannula while still hot, and allowed to cool before the cannula was inserted into the tail.

With both these substances results were obtained similar to those described in the case of agar-agar. There occurred an epidermal reaction, which was particularly marked over the ends of the tube, and which later subsided, a rapid and intense response of near-by wandering cells, sticking of leucocytes to the walls of near-by blood vessels very soon after the insertion of the tube, migration of leucocytes rapidly and in large numbers toward the tube, and collection of these cells around the tube, particularly at the open ends, followed by migration of leucocytes into the tube and eventual phagocytosis of the substances contained therein. This intense leucocytic reaction is shown in figure 5.

From these results it seems evident that such substances as agar-agar, gum arabic, and gelatin act as powerful stimuli for the migration of leucocytes. The leucocytic reaction called forth by these substances is similar in rapidity and intensity to that caused by the injection of semicooked starch grains. As in the case of the starch, the presence of these substances occasioned no response on the part of connective-tissue cells or of the endothelial cells of blood vessels or lymphatics other than the change in the blood vascular endothelium disclosed by the adherence of leucocytes to the vessel walls.<sup>1</sup>

<sup>1</sup> Since the completion of these experiments the work of Wolf ('21) has been published. This investigator made use of salt-free water solutions of agar as a carrying medium for testing the effect of a large number of chemicals on leucocytes in vitro. She found that only 4 per cent of the leucocytes used in the experiment were attracted toward the salt-free agar alone. We are unable to state whether the difference in these results is due to the different type of animals used in the two experiments (rabbits and dogs in Wolf's experiment), or to differences in the behavior of leucocytes in vitro compared to those found in the living animal, or to some differences in the preparation of the agar used in the two sets of experiments.



## DISCUSSION

It seems unnecessary at this time to refer to the numerous investigations which have been made upon the attraction of leucocytes toward different substances. Gabritchewsky ('90) classified various chemical substances as positively chemotactic, negatively chemotactic, and indifferent in their effect on leucocytes. Wells ('18) and his pupils, in recent studies, have tested many drugs in vitro and have added a long list to these three classes. Recent authors attribute the difference in the attraction of different drugs for leucocytes to surface tension phenomena: positively chemotactic substances being those which lower the surface tension of the cell, causing the cytoplasm to flow toward them. Moreover, Fenn ('21) considers phagocytosis to be a surface-tension phenomenon. In his study of the reaction of leucocytes to quartz and carbon particles, he found that the carbon was phagocytized three or four times as rapidly as the quartz, and he attributed the difference in rate to the more unstable condition of the carbon suspensions.

With the exception of the studies of Metchnikoff on the phagocytosis of carmine in the transparent tails of amphibian larvae, all these studies on the reaction of living leucocytes to different chemical substances have been made in vitro.

All of our observations on the reactions of cells to different substances injected into the transparent tails of tadpoles have been made in vivo on cells in their natural environment. Our earlier studies have shown that the different cell types respond in different ways to the various substances introduced into the fin. For example, lymphatic endothelium reacts to fatty substances by growing toward them, but not to starch grains; connective-tissue cells will take up carbon and carmine granules, but not starch grains or fat droplets.

With regard to the attraction of leucocytes toward the various substances used in our different experiments, we have noticed great differences in the degree of response.

Thus, paraffin oil proved to be practically an indifferent substance, since the reaction shown by the few wandering cells present near the site of injection was mild and transitory and no

greater than that produced by the simple insertion of the injection needle.

Next to paraffin oil may be placed foreign particles such as carbon or carmine granules and uncooked starch grains, which attracted wandering cells and, an hour or so later, leucocytes from near-by blood vessels in moderate quantities, which migrated toward the injected granules and proceeded to phagocytize them. These particles remained inside the cells indefinitely. The reaction which occurred after the introduction of small glass tubes, empty except for the surrounding chloretone solution, was essentially similar.

Fatty substances, such as small globules of olive oil, and oleic acid and emulsions of cream and yolk of egg, attracted leucocytes in larger numbers. The injected fat was taken up rapidly by the leucocytes and conveyed to near-by lymphatics, into which it was discharged in a soluble form.

In the case of inflammations produced by the injection of small globules of croton oil, leucocytes migrated from the blood vessels at some distance from the site of injury in greater numbers than in the case of the other substances just mentioned. The leucocytes did not phagocytize the croton oil, but became stationary at some distance from the injected globule, sending out processes until they came to resemble small fibroblasts. After the extrusion of the croton oil, they rounded up and later wandered away.

The most intense leucocytic reaction yet obtained was that produced by injecting starch granules, in a semicooked gelatinous form, and by the introduction of agar-agar, gum arabic, and gelatin, enclosed in small glass tubes. Leucocytes began to migrate through the walls of near-by blood vessels ten minutes after the insertion of these substances into the fin and their movement was very rapid. The leucocytes phagocytized the starch grains, while in the case of agar-agar, etc., they migrated into the ends of the tubes and eventually ingested all of the substances inside.

## SUMMARY

Starch grains introduced into the transparent fin expansion of the tadpole's tail produce different reactions according to the state of the starch, that is, according to whether it is uncooked, partially cooked, or fully cooked.

Uncooked starch granules attract a few leucocytes, which collect about the starch granules, and if the granules are not too large, phagocytize them. Such granules remain unchanged as cell inclusions, inside the leucocytes, for an indefinite period of time. The resistance to digestion of uncooked granules is probably due to their cellulose covering.

Cooked starch, in which all the granules have swollen and burst, so that most of the starch is outside the shells of the granules, dissolves rapidly, even before the arrival of many leucocytes, and undergoes a change into some other substance which does not stain with iodine. There is a moderate attraction for leucocytes, which evidently aid in the transformation of the starch. It is apparent that there are enzymes both outside and inside the leucocytes which act upon the cooked starch.

Semicooked starch granules exert an extreme attraction on leucocytes, which migrate rapidly and in large numbers toward such granules, completely surround them, engulfing them if small, and transform them, within the space of a few hours, into a substance which does not stain with iodine.

Agar-agar, gum arabic, and gelatin, enclosed in small glass tubes and inserted in the tail fin, provoke a reaction on the part of the leucocytes similar in kind and intensity to that produced by the semicooked starch granules.

No visible reaction on the part of the other subcutaneous tissues present in the vicinity of the injected substances—blood-vascular and lymphatic endothelium, nerves, and connective tissue—was observed in response to the presence of any of these substances.

It is a pleasure to have this opportunity to express to the authorities of the Marine Biological Laboratory, Woods Hole,

Massachusetts, where a part of this work was done, our appreciation of the facilities of the laboratory which were so generously granted.

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Abstracted by Robert Hall Bowen, author. Columbia University,  
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On the idiosome, Golgi apparatus, and acrosome in the  
male germ cells.

An attempt has been made to synthesize the results of recent investigations on the relation of the Golgi apparatus and idiosome to each other and to the acrosome in developing sperm cells. The idiosome in spermatocytes was originally defined by Meves on the basis of its supposed relation to the centrioles, whence arose the common confusion of the idiosome with a true 'attraction sphere.' It now appears that the idiosomic material is closely related to another cytoplasmic element, the Golgi apparatus. The latter may assume several appearances depending on the technique employed, but its condition in the living cell is probably that of a number of discrete rodlets. These may be clustered with the idiosomic material around the centrioles, or may be scattered through the cytoplasm without reference to the cell centers, in which case the idiosomic material is similarly distributed—a small mass to each Golgi element. The idiosome is thus to be defined by its relation to the Golgi apparatus rather than the centrioles. In the maturation divisions the Golgi elements are distributed to the spermatids by a complex dictyokinesis, and the idiosomic material probably accompanies the Golgi pieces in this distribution. In the spermatids the idiosome and Golgi apparatus may be reconstituted into a compact group (the familiar 'sphere' complex of many authors) or may remain in a scattered condition. The acrosome forms as a special differentiation product of this idiosome-Golgi complex, the exact method being characterized by great variation in detail in different animals, but fundamental similarity in essentials.

## ON THE IDIOSOME, GOLGI APPARATUS, AND ACROSOME IN THE MALE GERM CELLS

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### THREE FIGURES

The terms idiosome, Golgi apparatus, and acrosome have been rather generally applied in recent years to three characteristic structures of the male germ cells of animals, where they have proved a source of error and confusion for practically all students of spermiogenesis. Indeed, our understanding of these structures is now so beclouded that, even in recent text-books of cytology, they have usually been dismissed with a few casual comments, for the most part incorrect. The one point upon which there seems now to be fairly general agreement is that these three cytoplasmic components are definite and distinct parts of the cell, unrelated, in any immediate way at least, to mitochondria, chromidia, or other formed elements of the cytoplasm. In other words, the structures considered in this paper are to be thought of as parts of the cell comparable from the standpoint of morphological identity with the mitochondria, the centrioles, and perhaps even the nucleus. Recent studies have made it increasingly clear that the chief source of past errors is to be found in the technical methods which were formerly employed almost exclusively for the study of spermatogenesis. But since the problems of the cytoplasm have been attacked with a better appreciation of the fundamental difficulties involved, there has been an immediate clarification of many disputed points. My own interests happen to have centered in this field, and as a result of studies on the formation of the sperm in a variety of animals I have arrived at a fairly clear idea of what seem to me the fundamental features in the morphology of the idiosome, Golgi apparatus,

and acrosome in the male germ cells. I propose, therefore, in this paper to attempt a synthesis of my own and others' results, with a view to putting the available facts into a connected story which may serve as a supplementary chapter to the current text-book descriptions of spermiogenesis. That this account will prove to be correct in all respects is rather improbable, for our knowledge of the facts in different animal groups is still fragmentary and in many cases quite unsatisfactory; but I do hope to arrive at some scheme, satisfactory at least in a descriptive way, which may serve as a point of departure for further researches. No attempt will be made to treat the subject from an historical standpoint except in the interests of clearness, and illustrations will be drawn wherever possible from my own material, with which I am most familiar. The account will be limited to the conditions found in *typical, flagellate sperms*, since these are the only ones so far adequately investigated. I shall endeavor, also, to bring out particularly those features which still require further research for their elucidation.

#### THE SPERMATOCYTES<sup>1</sup>

When the testis of almost any animal (with the exception of many insects)—a mollusc or salamander, for example—is fixed in one of the orthodox cytological fluids, such as strong Flemming, and stained with Fe-hematoxylin and a counter-stain in accordance with the usual procedure, there will be found in the cytosome of each spermatocyte<sup>2</sup> a roughly spherical mass which takes the stain somewhat more darkly than the surrounding cytoplasm. Under good conditions, it is possible to demonstrate the centrioles within this mass, located centrally and usually in the form of paired granules (fig. 1A). To this spherical body Meves, in 1896, gave the name of *idiozom*,<sup>3</sup> a

<sup>1</sup> The acrosome, being characteristic of the developing sperm, is not of course present as such in the spermatocyte stages. This section deals, therefore, only with the idiosome and the Golgi apparatus.

<sup>2</sup> Primary spermatocytes are referred to unless specifically stated to the contrary.

<sup>3</sup> The usual English spelling is idiozome.



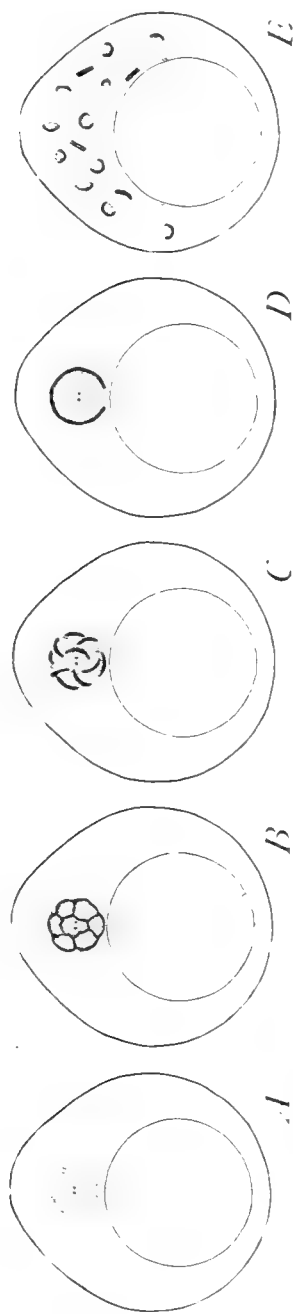


Fig. 1 Diagram to illustrate the structure of the idiosome and Golgi apparatus in the primary spermatocyte as demonstrated by various technical methods. Only the outlines of the nucleus and cytosome are indicated. The idiosome is stippled, and the centrioles are represented as simple granules. A, after the customary fixation with fluids containing acetic acid; B and D, frequent appearance after treatment by the impregnation methods for demonstrating the Golgi apparatus; C, particularly after chromeosomic fixatives with acetic acid absent or present in very small amount; E, scattered condition of the idiosome and Golgi apparatus in many inserts as demonstrated by many of the special technical methods for the Golgi apparatus.

term which was to stand "fuer die spezifisch beschaffene Huelle, welche die Centralkoerper in den maennlichen Samenzellen umgibt." Subsequently Meves ('03) suggested the substitution of *centrotheca*, a term which never succeeded in displacing its predecessor in general usage. More recently, Regaud ('10) has suggested the spelling *idiosome*, a change which has much to recommend it, and will be adopted in this paper. It is clear from Meves' remarks in adopting the term *centrotheca*, that he regarded the association of the idiosomic substance with the centrioles as the fact of primary importance—a view with which I cannot agree for reasons that will presently appear.

The real nature of the idiosome was from the very beginning obscure, and the fact that it was spherical and contained the centrioles immediately led to a most unfortunate confusion with the so-called *attraction sphere* of Van Beneden ('83) and the *archoplasm* of Boveri ('88). These latter terms were applied by their sponsors to the unusual protoplasmic differentiations which arise in connection with the centrioles during the division of certain animal eggs, but which, as has been repeatedly urged by various workers (for example, Duesberg, '20), have nothing whatever to do with the idiosome. The grounds for this distinction will appear more clearly in subsequent paragraphs. The point to be noted is that the terms *archoplasm* and *attraction sphere* have a very special and limited application, if indeed they have any very good basis in fact at all, and in any event are not to be used as indicative of any direct relationship with the idiosome.

This very simple condition of the idiosome<sup>4</sup> and its surroundings is characteristic of material which has been fixed in fluids containing a considerable amount of acetic acid. If the same kind of material (mollusc or salamander) be fixed in fluids in which acetic acid is absent or present only in small amount,

<sup>4</sup> I have not thought it worth while to include a discussion of the internal structural features which have been occasionally described in the idiosome. The meaning of these, and particularly their homologies, is as yet by no means clear. The reader who is interested in these features is referred to the papers of Papanicolaou and Stockard ('19) and Gatenby and Woodger ('21) on the idiosome of the guinea-pig.

such as Benda's modified Flemming or Flemming without acetic, the results are rather strikingly different. In a spermatocyte (stained with Fe-hematoxylin) from such a preparation (fig. 1C), the idiosome itself appears much as before, but closely applied to its periphery appear a number of small, crescentic rods which stain very sharply. These rods have of course long been known under a great variety of names (*Archoplasmaschleifen*, *Centralkapsel*, *Pseudochromosomen*, *formazioni periidiozomiche*, *Nebenkern*, etc.), since in certain animals—the molluscs particularly—they are often preserved in more or less perfect condition even after fixation in strong Flemming. They are, as Gatenby and others have recently shown, the representatives of the Golgi apparatus in the spermatocyte. The term apparatus is a very unhappy one, for in this case, as in so many others, the Golgi material apparently forms no connected *apparatus* at all. It would be in the interests of clarity to refer to this substance simply as the Golgi rods or some equivalent descriptive expression. It is important to note that these rods are actually separate units, and one does not get the impression that they are interconnected in any way by secondary fusions.

Again, in material prepared by one of the impregnation methods, for example, the silver reduction techniques of Golgi or Cajal, the idiosome and its surroundings may assume a somewhat different aspect. The idiosome substance itself may be more or less invisible, but its position is clearly marked by the Golgi apparatus which encloses it. The material of the Golgi apparatus is blackened intensely in successful preparations and has sometimes been described as forming a true network or reticulum, comparable to the classical figures of the '*apparato reticolare interno*' (fig. 1B). This appearance of the Golgi apparatus has been described by numerous authors as characteristic of many somatic cells after impregnation by silver nitrate or osmic acid, but the occurrence of the Golgi material in reticular form has rarely been described in spermatocytes. Perroncito ('10) thus describes and figures the Golgi apparatus in *Paludina* (a mollusc); but it should be noted that Gatenby

('18) has figured the Golgi apparatus in the same animal as made up of separate rodlets as in figure 1C.

Finally, after the same impregnation methods referred to in the preceding case, the Golgi apparatus may appear as a shell, closely applied to the idiosome and often (always?) incomplete on the side adjacent to the nucleus (fig. 1D). The shell itself may be more or less solid, or may appear as though made up "of a number of filaments of varying thickness." Duesberg ('20) has described such a structure for the Golgi apparatus in the spermatocytes of the opossum, and I have found more or less similar conditions in the salamander after silver nitrate (Cajal) impregnation. Furthermore, following osmic acid impregnation, I have found (salamander) that the 'shell' is sometimes entirely disrupted and the fragments scattered about in the cytoplasm in the vicinity of the idiosome.

How are these conflicting appearances of the Golgi apparatus to be harmonized; or, indeed, is there any reason to believe that the Golgi apparatus is always developed in the spermatocytes on the same structural plan? Unfortunately, we are not in position to give a categorical answer to these questions, chiefly because the majority of workers have so far been content to study the Golgi apparatus by very limited technical means. It is accordingly difficult to say in any particular case whether the results are descriptively correct or mere technical distortions of the truth. However, my own experience leads me to believe that the discrepancies in the published accounts of the Golgi apparatus, in the spermatocytes at least, are largely traceable to the untrustworthy results of the admittedly capricious impregnation methods (silver and osmic acid). Gatenby's work on *Paludina* and my own results on *Plethodon* (a salamander) seem to me to indicate unmistakably that the probable source of our difficulties is to be accounted for along the following lines. When the material is preserved in one of the chrome-osmic mixtures, which are generally agreed to be among the best-known cytoplasmic fixatives, the condition of the Golgi apparatus is almost invariably that of a cluster of rodlets applied to the surface of the idiosome (fig. 1C). After a

silver-nitrate impregnation the Golgi rods may undergo a slight disintegration resulting in their running together to form an apparent network or reticulum (fig. 1B), while still further disintegration or 'smearing' of the impregnation deposits may transform the Golgi apparatus into a more or less complete 'shell' (fig. 1D), or even disrupt the rods completely and scatter the fragments in the neighboring cytoplasm. Finally, after fixation in acetic-acid mixtures, the entire Golgi apparatus may be completely disintegrated and dissolved (fig. 1A). That the actual condition of the Golgi apparatus in the living spermatocyte is most nearly comparable to a group of *separate rodlets* is strongly supported not only by the general appearances which accompany what cytologists call 'good fixation,' but by the additional fact that fragmentary observations on living material seem to point in the same direction.<sup>5</sup>

It is clear from what has now been stated that the idiosome and the Golgi apparatus form in many spermatocytes a topographical unit, the center of which is occupied by the centrioles. Two questions at once suggest themselves: 1) is the close relation of the idiosomic substance and Golgi material merely a chance coincidence of location and, 2) what relation, if any, exists between these two materials and the centrioles, which would justify the retention of Meves' original definition of an idiosome and the consequent confusion with the attraction sphere and archoplasm?

It is possible to give at least a partial answer to these questions by reason of the unique conditions which obtain in many (perhaps all) insects. In the insects the idiosome seems sometimes to occur as a compact body in the very early spermatocytes,

<sup>5</sup> I wish to state specifically that this attempted explanation of the various appearances of the Golgi apparatus in the spermatocytes after different types of technique is *not* to be extended inconsiderately to conditions in somatic cells as well, in which the Golgi apparatus is most commonly described as having the form of a reticulum or network. The facts now known about the possible effects of technical treatment ought, however, to lead to a more critical scrutiny of the actual conditions in cells of all kinds. The facts suggest that in tissue cells, whose tenure of life is presumably a long one, the Golgi rods normally undergo more or less fusion resulting in the reticular condition so frequently described.

but eventually (in all known cases) and frequently (the Hemiptera furnish particularly good examples) from the very earliest spermatocyte stages onwards, the idiosome is represented only by many *separate* masses of idiosomic material each accompanied by a Golgi rodlet, the whole constituting a so-called Golgi body (Bowen, '20). These are at first definitely concentrated toward one pole of the cell and are thus directly comparable to the compact idiosome already considered. We are obviously dealing with an idiosome the constituent parts of which have become separated from one another. In the growth period the Golgi bodies begin to migrate away from their definitely polar position (fig. 1E), and eventually become scattered throughout the cytoplasm as I have described fully in the Pentatomidae (Bowen, '20). However, at no time is there any indication of a separation of the Golgi rodlets from the masses of idiosomic substance.

The behavior of the centrioles during these same stages offers a number of points of interest. In the very early primary spermatocytes the behavior of the centriole is fully known in comparatively few forms, but presumably the centriole from each spindle pole of the last spermatogonial division is always carried over directly into the succeeding primary spermatocyte. In the mollusc and salamander this centriole soon divides, but the parts remain close together until the maturation prophase are well advanced, and are located, as noted above, in the idiosome. In the Hemiptera, however, the centrioles behave very differently, for after dividing, the daughter centrioles begin at once to migrate around the nucleus (fig. 1E) until eventually they occupy diametrically opposite positions on the nuclear membrane. Meanwhile they each divide precociously in anticipation of the two rapidly succeeding maturation divisions. Throughout the migration of the centrioles and until the divisions of maturation begin, the Golgi bodies maintain no striking topographical relations to the centrioles.

The conditions in the insects furnish, it seems to me, an excellent basis for the interpretation of the idiosome structures as a whole. We are to think of the Golgi bodies (Golgi rodlet

plus idiosomic fragment) as the units of idiosome construction. These bodies tend, for some unknown reason, to collect in the vicinity of the centrioles, just as other parts of the cell (mitochondria, for example) may be polarized toward the centrioles when these bodies are together. If the centrioles remain thus together, the Golgi bodies likewise remain massed around them, giving rise to the familiar idiosome and Golgi apparatus complex of many authors. If, however, the centrioles separate, the polarization of the cell is apparently destroyed, and the idiosome fragments migrate out into the cytoplasm, together with the mitochondria, and become scattered throughout the cell in haphazard fashion. It becomes, therefore, perfectly clear that the important and primary relation is that which exists between the Golgi material and the idiosomic substance, while their joint relation to the centrioles is merely a topographic one. The basis for Meves' definition of the idiosome thus breaks down entirely and it is evident that we must look for a rigorous definition of the idiosome in terms of its interrelation to the Golgi apparatus rather than to the centrioles. I have suggested elsewhere (Bowen, '20) that this relation may possibly be of a permanent nature, the two components forming essentially a unit cell structure. Finally, this interpretation clears up much of the current misconception which obtains concerning the protoplasmic differentiations that sometimes surround the centrioles, such as the archoplasm, attraction sphere, centrosome, etc. These differentiations (so far as they have any existence in fact) have presumably a true organic relationship to the cellular centers, being connected with the processes of spindle formation, while the relation of the idiosomic substance to the centrioles is purely topographical and frequently does not obtain at all. Certain aspects of this interpretation will be further clarified by comparison with the following sections.

#### THE MATURATION DIVISIONS

It is not my intention in this paper to go extensively into the phenomena exhibited by the idiosome and Golgi apparatus during the maturation divisions, in part because the details

are as yet insufficiently known, but chiefly because the earlier workers almost invariably failed to observe any of the details by reason of the technique employed, and there is, accordingly, little to confuse the reader in the literature so far published. I wish, however, to indicate certain broad features of the division phenomena as an introduction to the spermatid stages, which have long been a source of inextricable confusion, due largely to ignorance of the fate of the idiosome and Golgi apparatus during the division phases.

From the facts now available it appears that there are two (possibly three) methods of distributing the Golgi apparatus in the spermatocyte divisions, the method being perhaps correlated with the previous condition of the idiosome. In the case of the idiosome which early loses its compact form (fig. 1E), the separate Golgi bodies undergo more or less fragmentation, prior to mitosis, which involves both the Golgi rods and the idiosomic substance. The resulting fragments (so-called dictyosomes) gradually collect around the poles of the spindle during the metaphase, each pole receiving an approximately equal quantity (Bowen, '20). During the telophase, when the centrioles are separating in preparation for the second maturation division, the dictyosomes are dispersed throughout the cell and are again accumulated around the spindle poles at the subsequent metaphase. During the telophase they may again become more or less scattered in the cytoplasm.

In the case of the idiosome which retains its compact form until immediately before the maturation divisions (figs. 1A to D), the end result is similar, but the intermediate steps are somewhat different. In this type, immediately preceding division, the idiosome and Golgi rodlets are divided into two groups which migrate with the centrioles to the spindle poles, where they are found at the metaphase just as in the preceding case (see, for example, Ludford and Gatenby, '21). Subsequently they become scattered throughout the cytoplasm and in the secondary spermatocytes are again assembled around the centrioles in compact form. The process is repeated in the second maturation division.



It is possible that a third type of division occurs in which the collection of the Golgi fragments around the poles is omitted, as Duesberg ('20) has described in the opossum. But the evidence for this is very scanty and further research may prove it quite erroneous.

In this brief outline of the division phenomena, I have purposely omitted mention of several important points which are still unsettled. The first of these involves the possible fragmentation of the Golgi rodlets prior to their accumulation around the centrioles at metaphase. A breaking-up or division of the Golgi rodlets certainly occurs in the Hemiptera which I have studied, and I have thought that there was good evidence of the same thing, in the salamander. Gatenby, on the other hand, insists that the Golgi rods are merely sorted out *intact*. The question is an exceedingly difficult one to settle on account of the inadequate technical methods now available, but it is one well worth further study. The second point in dispute has to do with the fate of the idiosomic substance during division. In the Hemiptera the dictyosomes are too small to analyze satisfactorily, and it is therefore impossible to say whether or not they retain the essential structure of the Golgi bodies through the division stages. From the fact that the idiosomic material can be found associated with the Golgi substance as soon as the dictyosomes have again fused to form aggregates sufficiently large to study accurately, I have concluded that in all probability each dictyosome is essentially a miniature Golgi body. Each Golgi fragment would thus be accompanied by a small mass of idiosomic material, and the distribution of both substances would accordingly be identical. No specific stain for the idiosomic material is at present known, and since it cannot be satisfactorily made out in small amounts, the question cannot be immediately solved. In the idiosome of the compact type, it seems certain that the substance accompanies the Golgi material to the spindle poles, but its subsequent fate is again obscured by the scattering of the Golgi pieces, and though it reappears with the latter around the centrioles in the late telophase, its exact intermediate behavior is uncertain. Ludford

and Gatenby ('21) believe that it becomes separated from the Golgi material in the interval—a view to which their figures do not lend particular support, and which to my mind runs counter to all the other available evidence.

It will be clear from the above that the exact behavior of the Golgi apparatus and the idiosome in the maturation divisions is very inadequately understood, but nevertheless that the Golgi material certainly, and the idiosomic material probably, is distributed to the resulting spermatids in a form identical, at least topographically, with the same materials of the original idiosome-Golgi complex of the spermatocytes. Furthermore, this opportunity may be taken to point out again that, as can be inferred from the above account, the idiosome has not been observed to take any part in the formation of spindle and asters during mitosis. This, I believe, is practically conclusive evidence that the idiosome is a cellular differentiation absolutely unrelated to attraction spheres or archoplasm, whose presumed function it is to provide material for the construction of the achromatic figure.

#### THE SPERMATIDS

At the close of the second maturation division the Golgi apparatus is present in each spermatid in the form of numerous, scattered pieces or dictyosomes. These may begin at once a process of fusion, so that the individual pieces become fewer in number, but larger in size. Presently they assume proportions not unlike the Golgi bodies of the growth period, and it is clear that each one is composed of the characteristic Golgi and idiosomic materials, arranged exactly as in the spermatocytes. According to the account of Ludford and Gatenby, the dictyosomes would perhaps undergo no fusion whatever, since they are, by description, complete Golgi bodies throughout the division stages. In any event, it is agreed that the Golgi apparatus and accompanying idiosome are usually present in the earliest spermatids in the form of more or less scattered Golgi bodies.

From this point on, however, the behavior of the Golgi bodies is subject to certain differences which may be conve-

niently classified under two characteristic types—types which recall the similar arrangements of the Golgi apparatus and the idiosomic material in the primary spermatocytes. These two types I propose to designate as follows: I, simple or fused type; II, compound or multiple type. These two types will be considered separately.

*Type I, Simple or fused*

In the great majority of animals thus far examined, the behavior of the Golgi bodies in the spermatid follows type I. In this type the Golgi bodies, formed as noted in the preceding paragraphs, gradually draw together, their idiosomic portions apparently fusing until eventually a compact mass is formed comparable to, and indeed directly homologous with, the characteristic idiosome of the primary spermatocytes as developed in the salamander and mollusc.

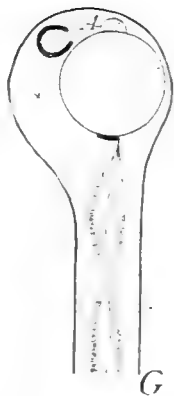
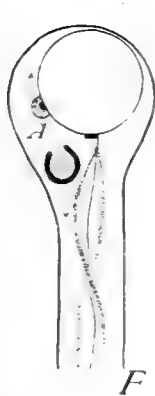
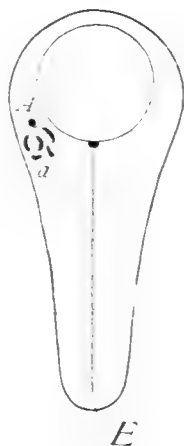
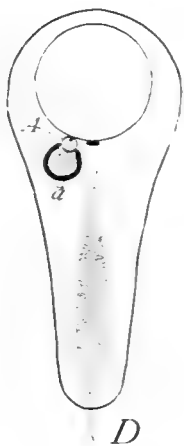
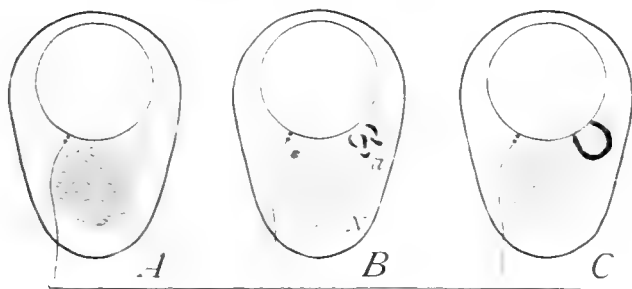
The mass thus formed may assume a variety of appearances exactly similar to those of figures 1A to D, and probably, in part at least, for the same reasons. Thus, in material fixed in acetic-acid mixtures the Golgi apparatus is completely dissolved, leaving only a spherical mass, the idiosome, or 'sphere' of many authors (fig. 2A). In material fixed especially in the chromosmium fluids with little or no acetic acid, the Golgi rodlets are sometimes clearly demonstrated, applied to the surface of the idiosome just as in the spermatocytes (fig. 2B). Very rarely a net-like Golgi apparatus comparable to that of figure 1B has been described (Perroncito, '10), but this is decidedly unusual. Very frequently, after impregnation methods, the Golgi material forms a solid shell enclosing the idiosomic substance, though here again the shell is incomplete on the side toward the nuclear membrane (fig. 2C). As a matter of fact, I have preparations of hemipteran testes in which, after the appropriate technique, all these various appearances (except the reticular condition of the Golgi apparatus) are clearly visible. The diagrams in figures 2A, B, and C are taken from these preparations. I had originally thought that the 'shell' condition shown in figure 2C represented the final step

in the fusion of the Golgi elements begun immediately after the second maturation division was completed. Recently, however, I have succeeded in demonstrating the presence (in *Euschistus*) of separate Golgi rods, as shown in figure 2*B*, so that the condition of figure 2*C* is perhaps to be explained as in part an artifact.

The Golgi apparatus-idiosome complex may be located in almost any part of the spermatid; but in insects, in which the mitochondria are aggregated into a solid, spherical mass or *nebenkern*, the idiosome is characteristically found near the angle between nucleus and *nebenkern*, and sometimes in a definite relation to the centrioles (figs. 2*A* to *C*). But the centrioles, it is almost universally agreed, are *never* located *within* the idiosome as is so characteristic of arrangements in the spermatocytes. Here, again, is a striking piece of evidence which tends to show that Meves' conception of the relation between idiosome and centrioles is erroneous. and that the relation between Golgi apparatus and idiosome is the essential thing.

The facts thus far elucidated show clearly the basis of many of the older descriptions of the spermatid idiosome. The acetic acid in the fixative, or other technical failings, caused the Golgi apparatus to be overlooked entirely during the spermatocyte divisions. The idiosome, once reconstituted in the spermatid, became visible again with the older methods, and was called an idiosome because it looked like its predecessor

Fig. 2 Diagram to illustrate the history of the idiosome and Golgi apparatus in the differentiation of the sperm. The idiosome and the mitochondria (*nebenkern*) are stippled; the centrioles and tail filaments are indicated, together with the outlines of nucleus and cytosome. *A*, acrosome; *a*, acroblast; *N*, *nebenkern*. *A*, *B*, *C*, typical early spermatid (Hemiptera; Bowen, '20), showing idiosomic conditions directly comparable to those of figures 1*A*, *C*, *D*; *D*, differentiation of the vesicular type of acrosome (Hemiptera; Bowen, '20); *E*, differentiation of the granular type of acrosome (Mollusca; Schitz, '16); *F*, deposition of the migratory type of vesicular acrosome (Hemiptera; Bowen, '20). The arrows indicate the direction of subsequent movement of the acrosome and acroblast (Golgi remnant); *G*, deposition of the stationary type of vesicular acrosome (*Ceuthophilus*; Bowen, '22); *I*, deposition of the stationary type of granular acrosome (Mollusca; Schitz, '16). In figures *G*, and *I* the arrow indicates the direction of subsequent movement of the acroblast.



in the spermatocytes of the same or some other animal. What was once a mere guess is now known to have the best of basis in fact. Furthermore, the appearance of this body near the nebenkern in insects was the obvious source of the long confusion which resulted from tracing the idiosome to spindle remnants, mitochondrial derivatives, and every other possible origin. The acrosome of the mature sperm is to be derived from the complex formed thus by the idiosome and Golgi apparatus, which I shall accordingly refer to henceforth as the *acroblast*. The *acroblast* is the exact equivalent of the idiosome plus Golgi apparatus of the spermatocytes.

The origin of the acrosome from the *acroblast* is essentially similar in all animals belonging to Type I, but the nature of the acrosome itself makes possible a subdivision on morphological grounds, as follows: Type I, vesicular; type II, granular.

The *vesicular* type of acrosome is of very common occurrence, appearing in typical form in many Mammalia, Hemiptera, and Amphibia. In this case the acrosome makes its first appearance as a small, clear, bubble-like vesicle<sup>6</sup> either within the idiosomic substance of the *acroblast* or on that part of its periphery from which the Golgi material is lacking. This vesicle gradually enlarges and soon projects conspicuously on one side of the *acroblast* (fig. 2D). The relation of the vesicle to the nucleus varies greatly in different forms, but the acrosome-*acroblast* complex seems in general to occupy some position near the spermatid nucleus. In the Hemiptera (fig. 2D) the vesicle is always in contact with the nuclear membrane; in *Locusta* (Otte, '07) the *acroblast* may be in contact with the nuclear membrane, while in *Ceuthophilus* (Bowen, '22) no particular relation seems regularly to occur. The ultimate size of the vesicle in proportion to the *acroblast* varies greatly in different animals, but the meaning of these differences is not known. Within the vesicle a darkly staining granule is characteristically developed, the granule being located typically on the inner wall

<sup>6</sup> In some animals several such vesicles may be produced simultaneously, the separate rudiments thus developed being subsequently merged to form a single vesicle.

of the vesicle at the point where it is in contact with the nucleus (fig. 2D). For convenience in description, I propose to call the vesicle, the *acrosomal vesicle*, and the granule, the *acrosomal granule*; together they form the *acrosome*.

The *granular* type of acrosome is of much less frequent occurrence than the vesicular type, having been worked out with any completeness only in Mollusca (see, for example, Schitz, '16, on *Columbella* and Gatenby, '19, on *Paludina*). In this type there is developed on the periphery of the acroblast a small, darkly staining granule, instead of the clear vesicle characteristic of the preceding case (fig. 2E). No definite relation between the nuclear wall and the acrosome seems to exist.

The homologies which presumably exist between the vesicular and granular types of acrosome are not at present evident, for we do not know whether the granular acrosome is to be compared with the vesicular acrosome as a whole, or only with the acrosomal granule which is developed within it. It is of course possible that the granular type may be produced by faulty differentiation, and that its fundamental similarity to the vesicular type is obscured by the stain. Furthermore, we do not understand in either case the exact source of the acrosome. Apparently it is produced in both cases by a differentiation of the acroblast material, but whether this involves both idiosome and Golgi apparatus, or is confined to the idiosome alone is not known. The important point to note is that the acrosome is a secondary product of the acroblast and that neither idiosomic nor Golgi material goes directly into its formation. After the acrosome is completely formed, the acroblast is separated from it (figs. 2F to I), gradually moves back through the cytoplasm of the sperm tail, and, after undergoing degenerative changes, is probably eventually cast out of the sperm in all cases together with other débris of spermiogenesis. After the separation of the acroblast from the acrosome, I have suggested (Bowen, '20) for it the name *Golgi remnant*, as a term of convenient description. The Golgi apparatus and idiosome are thus lost from the completed sperm, in which they are repre-

sented only by their differentiation product, the acrosome. Weigl ('12), and Gatenby and Woodger ('21), however, claim that in some mammals at least, a part of the Golgi material is left behind as a permanent contribution to the middle-piece of the sperm; but the exact status of this material is, I think, at present a matter of reasonable doubt.

The ultimate rôle of the acrosome is, in any case, to act as an apical piece for the sperm head. It must, in other words, be applied eventually to the anterior surface of the nucleus, this final location being attained, as a rule, not later than the earliest stages in the elongation of the nucleus (if any occurs) to form the sperm head. The method of deposition varies according to the location of the acrosome at the time when the acroblast is separated from it. On this basis, two types of acrosomal deposition can be recognized, as follows: Type I, migratory; type II, stationary.

In the *migratory* type the acrosome is developed at some point removed by a greater or less distance from the apical pole of the nucleus, and the acroblast is cast off with the acrosome still at some distance from its final place of fixation (fig. 2*F*). In this case the completed acrosome is left resting at some point on the nuclear wall, whence it migrates (as indicated by the arrow in fig. 2*F*) around the nucleus to its definitive position. This migratory type is especially characteristic of the Hemiptera (Bowen, '20), in which the vesicular type of acrosome occurs. I do not know of any examples of this migratory type in the granular type of acrosome.

In the *stationary* type, the acroblast and acrosome move together to the anterior surface of the nucleus. Here the acrosome is deposited in situ (figs. 2*G* and *I*), and the acroblast (Golgi remnant) is then gradually separated from it, behaving subsequently exactly as in the migratory type. Examples of the stationary type among vesicular acrosomes (fig. 2*G*) are quite common, those of the mammal (see, for example, Gatenby and Woodger, '21), *Ceuthophilus* (Bowen, '22), and probably the salamander (Bowen, '22) being representative examples. The molluscs present many examples of the stationary, granular type (fig. 2*I*) of acrosome (cf. Schitz, '16, and Gatenby, '19).



The exact fate of the acrosomal granule in the vesicular type is in most cases unknown, though it seems, in Hemiptera for example, to be related to a darkly-staining part which forms the tip of the acrosome. Acrosomes, particularly of the vesicular type, may undergo a great variety of differentiations, the case of *Locusta* (Otte, '07) being an especially good example; but the various shapes and structural features are not known to possess any points of general interest.

In figure 2 I have endeavored to represent in partially diagrammatic form the various types of behavior which occur in the simple or fused method of acrosomal formation. Beginning with a common type of acroblast (figs. 2A to C), either of two lines of development may be followed, leading to the production of an acrosome of either the vesicular or granular type (figs. 2D and E). The acrosome completed, it may either be deposited in situ or migrate to its definitive location subsequent to its deposition (figs. 2F, G and I).

#### *Type II, Compound or multiple*

The compound or multiple method of forming the acrosome is very incompletely known and seems, furthermore, to be of rather infrequent occurrence. At any rate, it has been observed very rarely, though this fact may be due to the much greater difficulties involved in its proper demonstration. In this type the Golgi bodies in the spermatid undergo little or no fusion (fig. 3), so that a single acroblast such as is figured in figures 2A to C is never produced. Instead, each of the Golgi bodies is apparently a small acroblast in itself—a probable interpretation which is borne out by the fact that in the fused type the acroblast is occasionally bipartite, each partial acroblast forming an acrosome proportional in size to the available material. The deposition of the partial acrosomes from these multiple acroblasts results presumably in the gradual building up of the complete acrosome. This process could undoubtedly be carefully analyzed in an animal which produced a vesicular type of acrosome from multiple acroblasts of sufficient size, and my observations on the Lepidoptera suggest that some of the moths may furnish material suitable for the purpose.

Thus far only two cases of multiple acroblasts are known, viz., the grasshopper (Bowen, '22) and the moths and butterflies.<sup>7</sup> In the grasshopper the acrosome is apparently of the granular type (fig. 3), but all the parts concerned are so minute that thus far the exact details of the formation of the acrosome have not been made out. The granular acrosome is very small and is formed near the base of the head, moving thence around the nucleus to its apical side just prior to the drawing out of

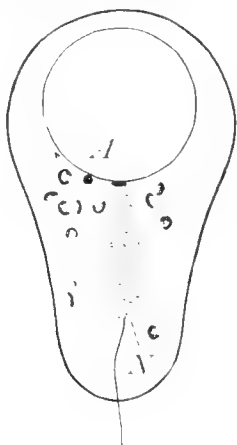


Fig. 3. Diagram to illustrate the formation of the acrosome from the multiple type of acroblast (acrididae; Bowen, '22). The arrows indicate the direction of the later movements of acrosome and acroblast. A, acrosome; N, nebenkern (mitochondria).

the sperm head. It thus belongs without question to the migratory type. In the *Lepidoptera* the acrosome is of the vesicular type, and is sometimes very large. In *Pygaera* the acrosomal granule itself is of extraordinary size and prominence. Here again the acrosome seems to be of the migratory type, but again the exact relation of the Golgi bodies to the parts of the acrosome has not been conclusively demonstrated. However, the multiple origin of the acrosome is clear, and the connection of the Golgi bodies with its formation is likewise beyond doubt. Thus far all my observations tend to bear out the

<sup>7</sup> The notes here given on the *Lepidoptera* are largely based on a study of *Pygaera* and *Callosamia*, the results of which I have not yet published.

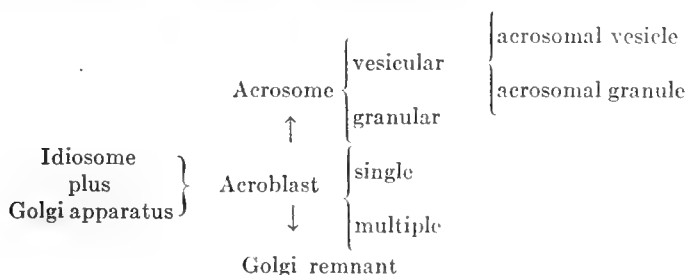
accuracy of the interpretation of the multiple type of acroblast which has been outlined above.

Aside from the single and multiple types of acroblast, an intermediate condition is an obvious possibility, but I am not acquainted with any form in which the facts are actually indicative of such a condition.

In the following table I have classified some of the better-known examples of acrosome formation according to the types which have been outlined in this paper.

GROUP	TYPE OF ACROBLAST	KIND OF ACROSOME	PLACE OF DEPOSITION
<i>Insecta</i>			
Hemiptera	Single	Vesicular	Migratory
Coleoptera	Single	Vesicular	?
Orthoptera			
Acrididae	Multiple	Granular	Migratory
Tettigoniidae	Single	Vesicular	Stationary
Lepidoptera	Multiple	Vesicular	Migratory
<i>Mollusca</i>	Single	Granular	Stationary
<i>Amphibia</i>			
Urodelia	Single	Vesicular	Stationary (?)
<i>Mammalia</i>	Single	Vesicular	Stationary

The nomenclature employed in this paper with reference to the spermatid structures may be outlined as follows:



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Resumen por el autor, E. R. Clark.

Reacciones de los capilares linfáticos aislados experimentalmente en la cola de las larvas de anfibios.

El autor ha separado porciones de los capilares del resto del sistema linfático, observando sus reacciones en el renacuajo vivo. Las porciones así separadas mantuvieron su vitalidad e identidad como tales linfáticos y eventualmente se reunieron con el sistema linfático. El tiempo transcurrido entre la separación y reunión ulterior con dicho sistema varía entre 14 y 21 días. Mientras están separados, los capilares aislados envían procesos y los retiran, absorbiendo y eliminando líquido, como demuestran los cambios en su calibre. No existe tendencia alguna a transformarse en otros tipos de células. Estos resultados prestan apoyo a la idea que supone al endotelio linfático como un tejido específico. Los vasos sanguíneos cortados se reunieron al cabo de tres días de separación. Los nervios cortados volvieron a reunirse al cabo de cinco días y las porciones separadas, que presentaban aspecto granular, bien pronto adquirieron su aspecto normal.

Translation by José F. Nonidez  
Cornell Medical College, New York.

## REACTIONS OF EXPERIMENTALLY ISOLATED LYMPHATIC CAPILLARIES IN THE TAILS OF AMPHIBIAN LARVAE

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TEN FIGURES

The normal growth of blood vessels and of lymphatics has been thoroughly studied by observation of the transparent tails of living amphibian larvae and the results reported elsewhere (E. R. Clark, '09, '12). By watching individual cells and vessels consecutively for days and even weeks, the method of growth of blood vessel and lymphatic capillaries, after their primary differentiation by sprouting, i.e., by extension from previously formed endothelium, has been firmly established. The manner in which the pattern of blood capillaries changes with changes in the circulation and the development of certain vessels of an indifferent capillary plexus into venules and arterioles are also problems which have been studied by direct observation of the living (E. R. Clark, '18).

In the course of these studies on blood vessels, an experiment was performed in which a blood capillary was completely isolated by cutting through its connections. Two days later this isolated capillary was found to have formed an anastomosis with one of the near-by circulating blood vessels, thus showing that a capillary which has become isolated does not lose its blood-vascular endothelial properties and may be reincorporated in the vascular system. This vessel was located near the margin of the fin in a region where active new formation of vessels was taking place.

This method of observation of a transparent region, such as the tadpole's tail, using chloretone anaesthesia to keep the animal motionless and an upright glass chamber such as has been

described (Clark, '12), seemed to afford a good opportunity for studying the regeneration of blood vessels and lymphatics, and also of nerves, after experimental injury.

#### DESCRIPTION OF EXPERIMENT

The following experiment was performed. Under the binocular dissecting microscope, a longitudinal cut was made in one of the tail fins of a Fowler's toad larva, anaesthetized with chloretone 1 to 3000. The cut was made with a sharp knife. Such a cut severed a number of blood capillaries, lymphatics, and nerves.

Immediately after the operation the tadpole was transferred to the observation chamber and the region studied under the compound microscope. It was found that the epidermal cells became opaque soon after the injury, drew together, and heaped up over the line of incision. Soon after making the cut, the processes of a number of the near-by connective-tissue cells became shorter and stiffer. Within fifteen minutes the leucocytes in the neighboring blood vessels began to adhere to the walls. Half an hour after the injury a distinct shrinkage of the corners of the cut was noticed. At this time also the leucocytes in the opposite fin near the region of the cut were observed to be sticking to the walls of the near-by blood vessels.

When a tadpole, operated on in this manner, was returned to fresh water and observed on the following day—approximately seventeen hours later—it was found that the cut surfaces had united, the epidermal layers having apparently healed by first intention, along the entire extent of the cut. A number of leucocytes had collected near the line of healing. Figure 1 is a low-power drawing of such a region on the day following a cut of this nature.

#### THE BLOOD VESSELS

When the region of a simple cut through the tail fin was observed on the day following the operation, a number of the blood vessels were found to end blindly near the line of healing. Observations showed a very slow circulation in the blood vessels



on the marginal side of the cut—the blood flowing for part of the time in one direction and then in the other. It should be noted that in this type of operation there were no entirely isolated capillaries as in the experiment reported above (Clark, '18), but instead a portion of a blood-vessel plexus was separated from another part with which it had been formerly connected (fig. 1).

On the following day the area of circulation had increased somewhat in the part of the plexus distal to the cut and the current flowed in a caudal direction.

On the next day—three days after making the incision—the cut blood vessels had all united with each other and the plexus was continuous, just as before the operation, and the circulation had been reestablished.

It is clear, then, from this experiment, just as was the case with the operation which isolated a single blood capillary, that when two parts of a blood-capillary plexus in amphibian larvae are separated from each other, the vessels reunite and the circulation is reestablished within the space of two or three days. The healing of such blood vessels, cut through in this manner, is accomplished by the same process of sprouting of the endothelium which characterized the normal process of growth in the tadpole's tail.

#### THE LYMPHATIC CAPILLARIES

The lymphatics cut through in this experiment were followed carefully and records made of them on successive days. Three days after the operation, when the severed blood vessels had all reunited, the cut lymphatics were still isolated. The proximal ends of these cut-off lymphatics were somewhat widened, while the remainder of the vessels were either normal in size or smaller than normal. During the succeeding days this widening of the proximal end of such an isolated lymphatic was noticed to be present at times, while at others the lumen was found to have returned to its normal caliber and, still later, the widening was again observed, thus showing that the fluid passes in both directions through the endothelium of such cut-off lymphatics. Aside from this change in the size of the lumen, no abnormalities

in the appearance or contour of such a cut-off lymphatic were noticed during the period of isolation. Such an isolated vessel, however, retains the power of growth and may even extend in a distal direction as was the case in the vessel recorded in figures 2 and 3.

Tadpoles operated on in this manner were followed for more than two weeks after the experiment with daily observations. At ten days after the operation, isolated lymphatics were still present and their appearance did not differ from that found soon after making the cut. In one such experiment a week or more after the operation the near-by lymphatic capillary with which the isolated vessel had formerly been connected began to send out a new sprout in the direction of the cut-off lymphatic. Such a sprout was at first solid and resembled exactly the sprouts of new growing lymphatic capillaries. On the twelfth day after the original cut, a fine thread from one of these new lymphatic branches had reached the isolated vessel and had united with it (figs. 2, 3, and 4).

In another specimen a lymphatic vessel was severed accidentally by the injection of a comparatively large globule of paraffin oil. Figures 5 to 10 are a series of camera-lucida sketches of this region, beginning with the thirteenth day after the injection (August 9th), when the lymphatic vessel (*B*) was still isolated. The lymphatic with which the isolated portion had originally been connected is shown at the top of the drawing and labeled *A*, but another lymphatic (*C*) had grown nearer to the cut-off vessel during the interval. During the subsequent days the nearer lymphatic (*C*) extended in the manner shown in figures 6 and 7. At first, as recorded on August 12th, the new sprout was apparently growing in a different direction instead of toward the isolated lymphatic (fig. 6). On the following day, however, this sprout was withdrawn and another one had grown out which approached nearer to the isolated vessel, while the isolated vessel itself was observed to send out a new sprout toward the approaching lymphatic outgrowth (fig. 7). On August 14th—eighteen days after the original experiment—these new sprouts from the two lymphatics had united (fig. 8). The connection

was at first narrow and solid, but during the succeeding three days it gradually enlarged, and finally, on August 20th (fig. 10), a continuous lumen was observed, and thus, twenty-four days after the original experiment which isolated it, this lymphatic capillary became completely reincorporated in the lymphatic system.

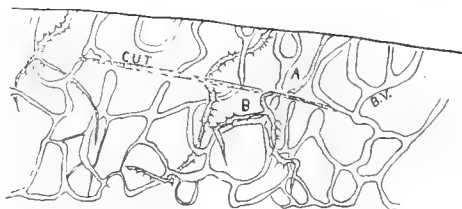
The picture of the formation of new sprouts, which were at first solid and later acquired a lumen, is identical with that found in the normal growth of lymphatic capillaries as described in earlier observations (Clark, '09, '12). It is evident from the present observations that lymphatic capillaries, experimentally isolated from the rest of the lymphatic system, retain their vitality and an independent power of growth for at least three weeks after the separation and are eventually reincorporated in the lymphatic system by a process which does not differ from the normal method of growth.

This cut through the tail also severed a number of peripheral nerves. The separated nerves became granular in appearance, with their finer processes invisible. On the fifth day following the experiment these nerves had reunited and recovered their normal appearance. However, further studies are necessary before a positive statement should be made as to the exact mode and extent of regeneration in these severed peripheral nerves of amphibian larvae.

#### DISCUSSION AND CONCLUSION

It has long been known that the severed capillaries of a blood-vessel plexus will regenerate and reunite across the gap made by an incision. A histological picture of the changes which occur in the case of healing of the blood vessels of the intestine in adult mammals has been given recently by Sabin ('20). She described the blood-vessel endothelium in such cases as resuming its embryological character and finds that solid angioblasts are formed from which blood vessels develop by a process of liquefaction of the protoplasm (cf. Halsted, '21).

The present study, made in the living transparent specimen, shows that when one portion of a circulating blood-vessel plexus



1



2

Fig. 1 Low-power sketch of the region of a cut through the dorsal fin of a tadpole, drawn eighteen hours after making the incision. The epidermal layers have healed. A number of blood vessels and lymphatics, cut across, end blindly at the line of healing. The lymphatics are dotted in this and succeeding figures. *B.V.*, blood vessel; *Lym.*, lymphatic; *A*, lymphatic vessel; *B*, isolated portion of lymphatic *A*.

Fig. 2 A small region from the same specimen shown in figure 1, drawn August 8th (five days after the original cut). The lymphatics shown here are those which were labelled *A* and *B* in figure 1. The proximal projection, present in the isolated lymphatic *B* on the day after the cut, has been withdrawn. The severed blood vessels have all healed and new blood capillaries have formed in the region.



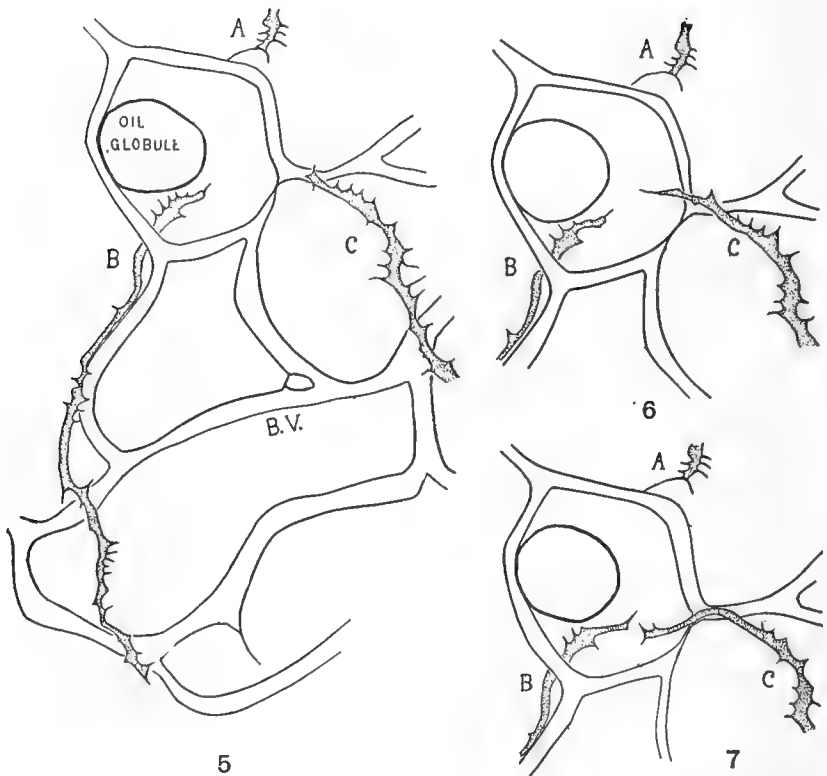


Fig. 3 Same region shown in figure 2, drawn August 13th (ten days after making the cut). Lymphatic *A* has extended toward the cut-off vessel. The isolated lymphatic *B* has extended in a distal direction.

Fig. 4 Same region drawn August 15th (twelve days after the beginning of the experiment). A solid sprout from the connected lymphatic *A* has extended and united with the isolated portion *B*.

Figs. 5 to 10 Series of camera-lucida sketches of a region in which a lymphatic vessel had been severed by injection of a globule of paraffin oil.

Fig. 5 August 9th (thirteen days after the injection). *A*, lymphatic from which isolated lymphatic *B* was separated; *C*, new lymphatic capillary which has grown into the region; *B.V.*, blood vessel.

Fig. 6 August 12th, lymphatic *C* has extended further.

Fig. 7 August 13th. New sprout from lymphatic *C* has been withdrawn and another one sent out which approaches nearer to the isolated lymphatic *B*, which in turn has sent out a new sprout toward the approaching capillary.

is experimentally isolated from another, the two portions reunite rapidly across the gap and the circulation is reestablished within a comparatively short space of time. Moreover, the former observation already referred to showed that a single blood capillary which has been completely cut off from the rest of the blood vascular system may be reincorporated in the system within

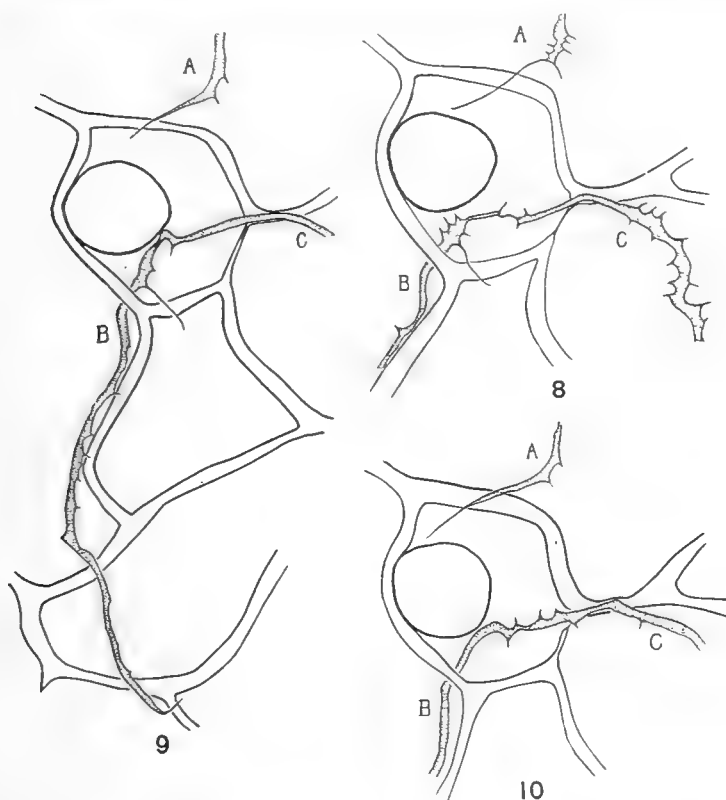


Fig. 8 August 14th (eighteen days after the separation of the lymphatic *B*). Shows a solid connection uniting the severed lymphatic *B* with the new sprout from *C*.

Fig. 9 August 16th. The connection between *B* and *C* is larger, but is still solid. The distal portion of the formerly isolated lymphatic is narrower than before (compare with fig. 5).

Fig. 10 August 20th (twenty-four days after the original experiment). A lumen is now present at the point of union of lymphatics *B* and *C*.

twenty-four hours after the experiment which isolated it. The method by which the blood capillaries reunite is identical with the process of normal growth of blood vessels, namely, by the sending out of solid protoplasmic sprouts from the blood vascular endothelium.

The exact mode of regeneration of lymphatic vessels has never been definitely established. Halstead ('21) in his recent article on elephantiasis, shows that the blood vessels have reunited across a gap within a few days and assumes that the lymphatics have also anastomosed in this space of time, but no injections were made to test this point. On the other hand, A. W. Meyer ('06) concludes from his investigations that lymphatics have no power of regeneration. Meyer ligated and cut the two largest lymphatic trunks anterior to the saphenous vein in four adult dogs and, in four other animals, he resected from 3 to 5 mm. of the trunk. The specimens were killed and examined fourteen to ninety-one days after the operation. No evidence of the regeneration of these lymph ducts was found, either by injection or by microscopic examination.

From the present experiment it is clear that lymphatic capillaries of amphibian larvae which are isolated experimentally from their connection retain their vitality and their power of growth for comparatively long periods of time (three weeks and more) and are eventually reincorporated in the lymphatic system. During their period of isolation there is evidence of the passage of fluid in both directions through the endothelial wall. The regeneration, or better, the healing of such severed lymphatic capillaries is very much slower than in the case of blood-vessel capillaries separated in a similar manner.

These studies bring new evidence in favor of the specificity of blood-vessel and of lymphatic endothelium, for they show that both of these types of endothelium remain specific even when completely isolated from the remainder of their respective systems. It was shown in a previous paper that an isolated blood capillary remains specific and reunites with the blood vascular system. In this study it is found that lymphatic capillaries when isolated retain their specific modes of reaction and reunite



with the lymphatic system. There is no indication of the transformation, at least at this stage and in this animal, of blood-vessel into lymphatic endothelium or vice versa, nor of either type of endothelium into indifferent mesenchyme cells or wandering cells.

The suggestion made by F. T. Lewis ('06) that, in very early stages, blood vessels may become cut off and transformed into lymphatic endothelium—an hypothesis which has been neither proved nor disproved—still remains a possibility for earlier stages. However, it seems to the author that this study makes that possibility a little less probable.

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Resumen por le autor, A. Kuntz.

Sobre la existencia de arcos reflejos en los plexos  
mientérico y submucoso.

El presente estudio se basa primariamente sobre preparaciones del estómago e intestino del gato, teñidas con el azul de metileno. Las fibras preganglionares viscerales eferentes y las que se originan en el plexo submucoso establecen sinapsis con las neuronas del plexo mientérico. Las sinapsis de dos neuronas en el mismo ganglio y las de dos neuronas en ganglios separados del mismo plexo existen también en el plexo mientérico y en el submucoso. Las sinapsis de neuronas de los plexos entéricos indican la existencia de arcos reflejos locales. El presente estudio no suministra pruebas de la existencia de redes nerviosas en las paredes del tubo digestivo. Las fibras que se originan primariamente en el plexo submucoso terminan en el epitelio gástrico e intestinal. Estas fibras son probablemente las dendritas de neuronas del plexo submucoso. Los hallazgos anatómicos mencionados suministran una base estructural para la interpretación racional de todas las actividades usuales del estómago y del intestino, con la excepción de la contracción rítmica. El autor llama la atención hacia los trabajos de Magnus, Gunn y Underhill, y Alvarez y Mahoney, los cuales indican la naturaleza miogénica de estas contracciones.

Translation by José F. Nonidez  
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## ON THE OCCURRENCE OF REFLEX ARCS IN THE MYENTERIC AND SUBMUCOUS PLEXUSES

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NINE FIGURES

### INTRODUCTION

The myenteric and submucous plexuses constitute one of the most obscure portions of the sympathetic nervous system. Much has been written about them, but relatively few anatomical investigations have dealt with them intensively. The morphological characters of the ganglia and fibrous commissures, including the histological structure of the neurons, composing these plexuses have been described in detail. However, the available anatomical data give us little exact knowledge regarding the interrelationships of the neurons or of the relationships of the latter to the general visceral efferent and afferent neuron systems. The present status of our knowledge regarding the enteric plexuses is well characterized by Ranson ('21) when he says, "since next to nothing is known about the structure of these plexuses and the interrelations of the nervous elements which compose them, the problems relating to the control of the movements of the alimentary tract are very baffling."

Ranson ('21) has recently reviewed the literature bearing on the functional relationships of the enteric plexuses, including references to the more important anatomical studies. Erick Müller ('21) has also reviewed the literature bearing on the microscopic structure of these plexuses. Therefore, a systematic review of the literature will not be undertaken in this paper.

The myenteric plexus is composed of numerous somewhat irregular, flattened or lens-shaped ganglia interposed between the longitudinal and circular muscle layers and connected with

each other by commissures of non-medullated fibers. The submucous plexus comprises numerous small ganglia located in the submucous layer and, likewise, connected with each other by commissures of non-medullated fibers. Similar commissures also connect the myenteric and submucous plexuses with each other. Non-medullated fibers may also be traced from the ganglia in the myenteric plexus into the longitudinal and circular muscle-layers where they terminate on muscle fibers. Likewise, non-medullated fibers may be traced from the ganglia of the submucous plexus into the muscularis mucosae, among the gastric and intestinal glands, and into proximity with the gastric and intestinal epithelium.

These general morphological characters of the myenteric and submucous plexuses have been described repeatedly. Therefore, a detailed description would be superfluous at this time. It is the writer's purpose in the present paper to set forth anatomical evidence of the occurrence of local reflex arcs within the walls of the stomach and intestine and to point out, as far as possible, the anatomical basis for a rational interpretation of the physiological activities of these organs.

#### METHODS

The observations set forth in the present paper were made almost exclusively on methylene-blue preparations of the stomach and small intestine of the cat. The writer has during the past ten years repeatedly attempted, by the use of both intravital and impregnation methods, to secure good histological preparations of the enteric plexuses, but with only indifferent success. The preparations most servicable for the study of the morphology of the neurons were secured by the use of the pyridine-silver method. Those most servicable for the study of synapses within the enteric plexuses and fiber terminations on muscle, gland, and epithelial cells were secured by the intravital methylene-blue method.

## MICROSCOPIC STRUCTURE

*Morphology of neurons*

Classifications of the neurons in the enteric plexuses, as well as in other portions of the sympathetic nervous system, according to morphological types have been attempted repeatedly. Dogiel ('95-'99) described neurons of two distinct types in these plexuses. Those of type I were characterized by numerous short, frequently branching dendrites and an axon which could be traced into the musculature. Those of type II were characterized by few long, infrequently branching dendrites and an axon similar in appearance to the dendrites. Dogiel interpreted the neurons of type I as motor and those of type II as sensory in character. As early as 1889 Cajal discovered, in addition to the larger neurons in the ganglia in the walls of the digestive tube, certain small spindle-shaped and angular cells with varicose processes which occur in all the layers and are not limited to the ganglia. He designated these elements as interstitial cells, but interpreted them as free neurons. La Villa ('97, '98) studied these elements in methylene-blue preparations and also interpreted them as neurons. In his later work Cajal ('09) still maintained that the interstitial cells are nervous elements and emphasized the presence in them of neurofibrillae, as shown in material prepared by silver-impregnation methods. Dogiel ('95) interpreted these interstitial cells not as neurons, but as connective-tissue elements. Both Heidenhain ('11) and Huber ('13) concur in this interpretation. L. R. Müller ('11) could differentiate two types of neurons in the myenteric, but not in the submucous plexus. In the former plexus the neurons of the one type lie free in small lacunae and give rise to numerous processes. Those of the other type lie close to the musculature and give off small dendrites to it. He suggests that the neurons of the former type may be sensory and those of the latter type motor. However, he clearly points out that there are no data available which warrant a positive conclusion regarding functional differences in these neurons. Erick Müller ('21) claims to have substantiated the conclusion of Dogiel that the neurons in the myenteric and sub-

mucous plexuses conform to two morphological types. Furthermore, he identifies the interstitial cells of Cajal with the neurons of type II of Dogiel. However, he does not accept Dogiel's interpretation of the functional relationships of the neurons of this type.

The present study contributes little to our knowledge of the general morphology of the neurons in the enteric plexuses. There is a wide range of variation in the size, form, and other characters of the neurons in both plexuses. The average size of the neurons in the myenteric plexus is somewhat greater than the average size of those in the submucous plexus. Both neurons with short, frequently branching dendrites and neurons with longer, less frequently branching dendrites occur in the myenteric plexus. Probably neurons of both types occur also in the submucous plexus. In the latter plexus, neurons with relatively long dendrites clearly preponderate. However, many neurons in both plexuses conform closely neither to the one nor to the other of these types. The present study does not warrant the conclusion that the neurons in these plexuses can be classified according to two distinct morphological types. Regarding the interstitial cells, the writer must agree with those authors who have interpreted them as connective-tissue elements. These elements are brought out more clearly in the pyridine-silver than in the methylene-blue preparations used in this study. They are especially abundant in the submucous layer. They differ so widely, both in their staining reactions and in their structure, from the neurons in the ganglia that the conclusion that they are nervous elements seems unwarranted.

### *Synaptic relationships*

The problems regarding the interrelationships of the neurons in the enteric plexuses and their relationships to the general visceral efferent neuron system are of primary importance. Available anatomical data throw little light on them. Synapses within the walls of the digestive tube have rarely been observed. The writer described such synapses in an earlier paper (13).

However, the fibers whose terminations in relation to neurons were observed could not be traced from their origin; consequently, they could all be interpreted as preganglionic fibers. Some of the methylene-blue preparations used in the present study are especially favorable for the study of synapses by reason of the unequal reaction of the neurons to the stain. In these preparations some of the neurons are stained intensely, while others are stained very lightly or not at all. In many of the ganglia but few of the neurons present are apparent. The terminal portions of the axons commonly react intensely to the stain; consequently, synapses may be observed most readily on neurons which are stained very lightly.

The synapses observed in this study are all similar in character. As the axon approaches the neuron in relation to which it terminates, it divides into several branches which embrace the cell body. These branches give rise to smaller branches with which they form a more or less complex pericellular arborization. In many instances the source of the axon involved in the synapse cannot be determined. In others the source of the axon may be inferred from the position and relationships of the commissure in which it approaches the ganglion where the synapse occurs. In still others the synaptic relationship involves two neurons located in the same ganglion.

The synaptic relationships indicated above are illustrated in the accompanying figures. Figure 1 is a camera-lucida drawing illustrating several synapses in a small ganglion in the myenteric plexus in the small intestine. The fibers which are shown entering this ganglion are contained in a commissure which connects it with a small ganglion in the submucous plexus. Doubtless, they arise in the latter ganglion. Axons *a* and *b* either represent preganglionic visceral efferent fibers or axons which arise in another ganglion in the myenteric plexus. Figure 2, likewise, is taken from a longitudinal section of the small intestine. It illustrates a similar synapse involving an axon which probably arises in the submucous plexus and a neuron in the myenteric plexus. Synapses which clearly involve axons which arise in the myenteric plexus and neurons in the submucous plexus were

not observed. However, synapses occur also in the submucous plexus. Figure 3 is a camera-lucida drawing illustrating two synapses in approximately exact spatial relation to each other and to the muscularis mucosae in the stomach. Inasmuch as the axons involved in these synapses run nearly parallel with the muscularis mucosae as far as they may be traced and approach the respective neurons in opposite directions, both cannot be interpreted as preganglionic visceral efferent fibers. Probably both synapses involve only neurons in the submucous plexus. Synapses involving two neurons located in the same ganglion in the myenteric plexus are illustrated by camera-lucida drawings in figures 4 and 5. Synapses involving two neurons located in the same ganglion in the submucous plexus are illustrated in the same manner in figures 6 and 7. In all these cases the axon may be traced without interruption from its origin to its termination. The neuron from which the axon arises is stained intensely; the one in relation to which it terminates is stained very lightly. The majority of the neurons in the ganglia are stained very lightly or not at all. These conditions render the preparations

Fig. 1 Camera-lucida drawing illustrating synapses in a ganglion in the myenteric plexus in the small intestine of the cat, longitudinal section. Peripheral line indicates the periphery of the ganglion. *a* and *b*, probably preganglionic visceral efferent fibers; *c*, fibers arising in the submucous plexus.

Fig. 2 Camera-lucida drawing illustrating synapse of a fiber arising in the submucous plexus with a neuron in a small ganglion in the myenteric plexus in the small intestine of the cat, longitudinal section. Peripheral line indicates the periphery of the ganglion and its relation to a commissure connecting it with the submucous plexus.

Fig. 3 Camera-lucida drawing illustrating synapses (*a* and *b*) in the submucous plexus in the stomach of the cat in approximately exact spatial relation to each other and to the muscularis mucosae (*m*).

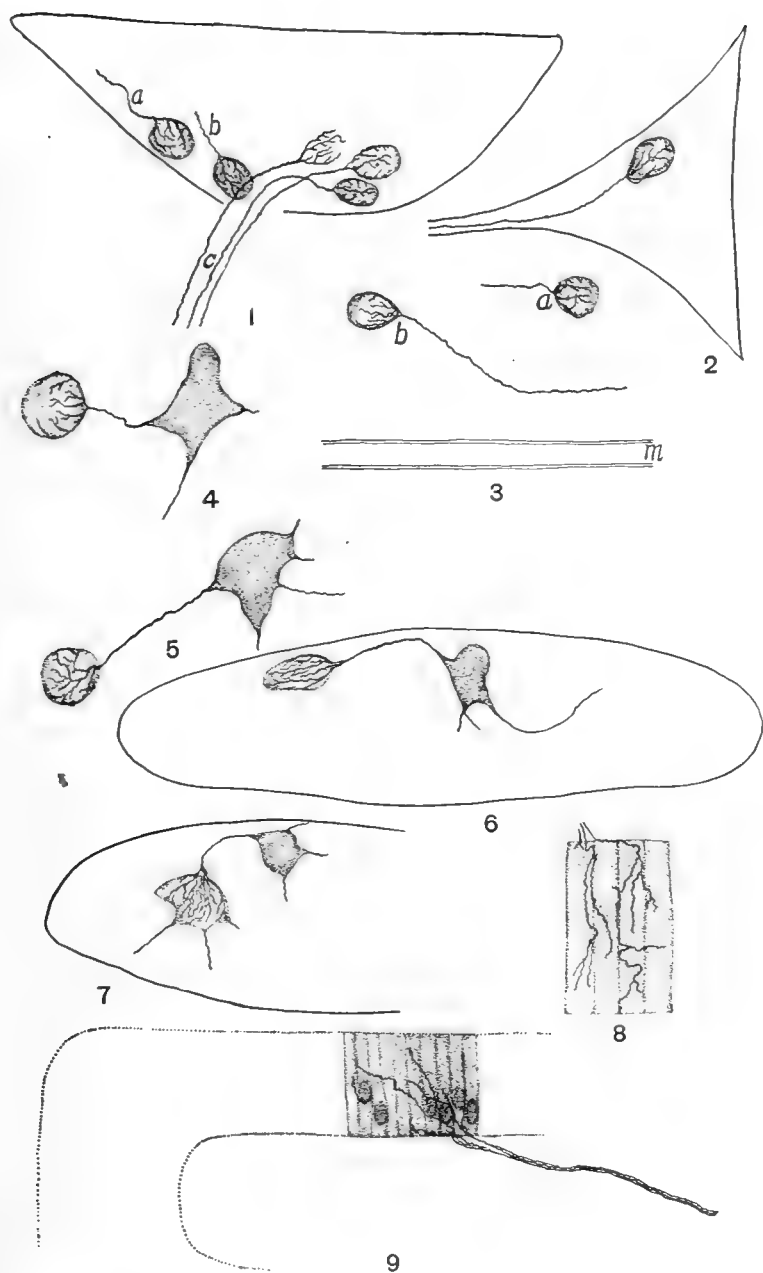
Figs. 4 and 5 Camera-lucida drawings illustrating synapses involving two neurons in the same ganglion in the myenteric plexus in the small intestine of the cat.

Figs. 6 and 7 Camera-lucida drawings illustrating synapses involving two neurons in the same ganglion in the submucous plexus in the small intestine of the cat. Peripheral lines indicate the peripheries of the ganglia in longitudinal section.

Fig. 8 Camera-lucida drawing illustrating terminations of sympathetic fibers in the intestinal epithelium at the distal end of a villus.

Fig. 9 Camera-lucida drawing illustrating terminations of sympathetic fibers in the intestinal epithelium near the distal end of a villus.





very favorable both for the observation of the uninterrupted axon and its terminal arborization.

The synaptic relationship described above, which involves an axon in a commissure which connects the submucous with the myenteric plexus and a neuron located in a ganglion in the latter plexus, strongly suggests a local reflex arc of which the afferent element is a neuron in the submucous plexus. Obviously, two neurons which are located in the same ganglion and sustain a synaptic relationship to each other cannot be interpreted as the terminal elements in a visceral efferent chain. They can only be interpreted as constituting a partial or complete local reflex arc. Possibly such reflex arcs involve a third neuron. The data at hand afford no positive evidence on this point. The conditions stated above which make possible the observation of synapses would practically preclude the observation of two successive synapses in an arc composed of three neurons, even though such arcs were known to be present. Whether these local reflex arcs comprise two or three neurons is a matter of minor importance. The synaptic relationships involving two neurons, as described above, demonstrate a nervous mechanism within the walls of the stomach and intestine through which local reflexes may be carried out.

#### *Fiber-terminations*

Terminations of sympathetic fibers on muscle and gland cells in the walls of the digestive tube have been described repeatedly. Their occurrence is generally admitted. Terminations of sympathetic fibers in the gastric and intestinal epithelium have also been observed. Such fiber-terminations were described by Sacussew ('97) in the intestinal epithelium of the fish and by R. Müller ('08) in the gastric epithelium of the frog. The writer ('13) also described fiber-terminations on the gastric and intestinal epithelium of the cat. More recently, Erick Müller ('20, '21) described fiber-terminations in the digestive epithelium in *Selachii* and in the chick. Nevertheless, the present attitude of the majority of anatomists regarding the occurrence of terminations of sympathetic fibers in the gastric and intestinal epithelium seems to be one of scepticism.

The preparations used in the present study admit of no doubt regarding the occurrence of sympathetic fiber-terminations in the gastric and intestinal epithelium in the cat. Non-medullated fibers penetrate the muscularis mucosae and descend into the gastric folds and plicae and the intestinal villi in large numbers. Many of these fibers ramify among the glands in the mucosa, others supply the intravillus muscle fibers. However, many of them approach the epithelium and give rise to terminal branches which ramify among the epithelial cells. Camera-lucida drawings of such fibers in relation to the epithelium are presented in figures 8 and 9. Fiber-terminations in the gastric and intestinal epithelium were described by the writer in essentially the same manner in the earlier paper referred to above. However, it is now clear that the terminal branches of such fibers ramify more deeply among the epithelial cells and approach the free ends of the latter more closely than they could be traced in the earlier study. Obviously, it is impossible in sections to trace individual fibers from their cells of origin in the enteric plexuses to the gastric or intestinal epithelium. However, individual fiber bundles may be traced from the submucous plexus into proximity with the epithelium where certain of the fibers give rise to terminal branches which ramify among the epithelial cells. In view of the current teaching, it might be argued that the fibers which terminate in the gastric and intestinal epithelium are not sympathetic, but general visceral afferent fibers. Clearly, their identity with the latter cannot be demonstrated. Furthermore, they are present in such abundance that they could not be accounted for as visceral afferent fibers contained in the vagus and spinal nerves, even though we should admit of the most profuse branching. Neither can they be differentiated histologically from the other fibers which penetrate the muscularis mucosae. The majority of the fiber bundles involved also arise in the submucous layer. Doubtless, the fibers which ramify among the gland cells represent axons. Those which approach the epithelium present the same histological appearance; however, inasmuch as they terminate in the epithelium, they are, doubtless, afferent in character and probably represent dendrites.

## DISCUSSION

According to current anatomical teaching, the sympathetic nervous system is essentially an efferent system. The visceral efferent chain comprises a preganglionic neuron located in the central nervous system and a postganglionic neuron located in a sympathetic ganglion. Afferent visceral impulses which are mediated by the general and special visceral afferent neurons may be transmitted to the preganglionic visceral efferent neurons in the cranial and spinal medulla and result in visceral reflexes. The occurrence of visceral reflex arcs which comprise only sympathetic neurons is not generally admitted.

There are neither anatomical nor physiological data available which either suggest or demand the presence of afferent sympathetic neurons in any part of the sympathetic nervous system except the plexuses in the walls of the hollow viscera. Furthermore, exhaustive anatomical studies of other parts of the sympathetic system have failed to reveal synaptic relationships except those of preganglionic visceral efferent fibers to sympathetic neurons. On the other hand, there is a large volume of physiological data available regarding the activities of the digestive tube which cannot be intelligently interpreted unless we admit the occurrence of afferent sympathetic neurons in the enteric plexuses. Consequently, some physiologists have disregarded the current anatomical teaching and have assumed the presence of local reflex arcs within the walls of the digestive tube.

As stated above, Dogiel described sympathetic neurons of two distinct morphological types which he interpreted, respectively, as motor and sensory in character. He believed that neurons of both types occur in all parts of the sympathetic nervous system. As pointed out above, there are no facts available which indicate the occurrence of sensory (afferent) sympathetic neurons in any part of the sympathetic system except the plexuses in the walls of the hollow viscera. Neither have the later attempts of Cajal ('05, '06), Michailow ('08, '11), and others to classify sympathetic neurons according to morphological types resulted in any notable advances in our knowledge regarding their functions and interrelationships. The fact that certain investigators,

especially Michailow, have described sympathetic neurons of more than two morphological types only emphasizes the diversity of these elements and indicates that there are many sympathetic neurons which conform closely neither to type I nor to type II of Dogiel. Therefore, the correlation of motor and sensory functions with distinct morphological characters of sympathetic neurons seems unwarranted.

There has long been a tendency on the part of certain investigators to regard the enteric nervous mechanism as composed, at least in part, of nerve nets characterized by actual continuity of protoplasm between the constituent cellular elements. Bethe ('03) and R. Müller ('08) described the enteric plexuses in the frog as consisting of true nerve nets. These authors probably did not regard these plexuses as differing fundamentally from other parts of the sympathetic nervous system. They were still unconvinced of the validity of the neuron theory. Erick Müller ('20) described the enteric plexuses in the *Selachii* as composed exclusively of nerve nets. In a later paper ('21), he described these plexuses in birds and mammals as consisting in part of nerve nets and in part of free neurons. Obviously, this author regards the enteric nervous mechanism as differing fundamentally from the other parts of the sympathetic nervous system. He says the cells in the enteric plexuses are cells *sui generis* ("Die Elemente der Darmgeflechte sind Zellen *sui generis*"). He emphasizes the presence in the enteric plexuses of neurons of both type I and type II of Dogiel, but does not agree with Dogiel that neurons of type II occur in the ganglia of sympathetic trunks and the prevertebral plexuses. Furthermore, he identifies the interstitial cells of Cajal, which, as pointed out above, were regarded by Dogiel and Huber as connective-tissue elements, with the neurons of type II of Dogiel.

Müller's conception of the morphology and functional relationships of the enteric plexuses is further conditioned by his conception of the development of these plexuses. He maintains that the neurons of type I are of vagus origin, while those of type II are of sympathetic origin, i.e., the cells which give rise to neurons of type I are displaced into the walls of the digestive

tube along the paths of the vagi, while those which give rise to neurons of type II are displaced from the sources of the cells which give rise to the ganglia of the sympathetic trunks. Consequently, he assumes that the neurons of type I remain functionally associated with the vagi and those of type II with the sympathetic nerves; i.e., the former subserve motor, the latter inhibitory functions. Both myenteric and submucous plexuses, according to Müller, comprise both free neurons and nerve nets. He further states that the myenteric plexus comprises only vagus elements in the stomach and both vagus and sympathetic elements in the intestine, while the submucous plexus, throughout the stomach and intestine, comprises only sympathetic elements. Müller assumes that conduction in the walls of the digestive tube is accomplished by the nerve nets, while local reflexes must be interpreted as axon reflexes.

As pointed out above, there is no secure basis for the classification of neurons in the enteric plexuses according to two distinct morphological types. Even though Müller's classification could be accepted, his contention that the neurons of type I are of vagus and those of type II of sympathetic origin could not be established. As shown by the writer in a series of earlier papers, the enteric plexuses arise primarily from cells which advance peripherally along the vagus nerves. This finding has been corroborated by Abel ('12) and Stewart ('20) and has recently been verified experimentally by the writer ('22). Müller admits that his findings regarding a contribution of cells of sympathetic origin are not so trustworthy as might be desired when he says, "das Verhältnis des sympathischen Anteiles habe ich nicht so genau untersuchen können" (p. 241). We do not maintain that no cells of sympathetic origin become incorporated in the enteric plexuses. However, there is no reason to assume that such cells would differ essentially either from the cells of vagus origin or from those which remain in the sympathetic trunks and the prevertebral plexuses. Furthermore, cells of vagus origin are present in the submucous plexus before pathways are established along which cells of sympathetic origin could be displaced into the walls of the digestive tube. Therefore it,

could not be maintained that the submucous plexus comprises only neurons of sympathetic origin. If a distinct contribution to the enteric plexuses of cells of sympathetic origin which differ essentially from the cells of vagus origin cannot be established, there is, of course, no basis for Müller's theory of the functional relationships of these plexuses.

The present study affords no evidence of the occurrence of true nerve nets in any part of the digestive tube. Obviously, negative findings cannot disprove the occurrence of nerve nets. However, the finding of numerous synapses, especially synapses which involve two neurons in the same ganglion, lends strong support to the theory that these plexuses comprise only free neurons.

Our knowledge regarding the effects of vagus and splanchnic stimulation and the normal gastric and intestinal reflexes involving these nerves is much more complete than our knowledge regarding the rôle of the enteric plexuses. However, it is generally conceded that the control of the movements of the stomach and intestine is exercised largely by the myenteric and submucous plexuses, although many of the movements whose essential features are determined by the local nervous mechanism may be modified and regulated through the extrinsic nerves.

The known reflexes of the alimentary canal have recently been summarized by Ranson ('21). Many of these reflexes involve extrinsic nerves, others are carried out by the intrinsic nervous mechanism. As indicated by experimental observations, of which a few are referred to below, many of the normal activities of the stomach and intestine involve the more or less independent functioning of the enteric plexuses. According to Cannon ('97, '11), the intermittent discharge of food from the pylorus is controlled by the local nervous mechanism. According to Carlson ('16), the essential features of the hunger contractions are determined by the local neuromuscular mechanism, although these contractions may be modified and regulated by extrinsic nerves. All the movements of the intestine persist after the extrinsic nerves are severed. According to Cannon ('12), the myenteric reflex is responsible for peristalsis in the small intestine,

but not for gastric peristalsis nor for antiperistalsis and segmentation in the intestine.

All the phenomena noted above and others which might be mentioned seem to require a local reflex mechanism within the walls of the stomach and intestine. On the other hand, the rhythmic contractions of the stomach and intestine persist following the administration of nicotin in doses sufficient to prevent conduction through synapses. Furthermore, conclusive anatomical evidence of the occurrence of synapses in the enteric plexuses, except those of preganglionic with postganglionic efferent neurons, was not forthcoming. Consequently, many physiologists as well as anatomists have not been willing to admit the occurrence of local reflex arcs in the enteric plexuses. Doubtless, these circumstances are largely accountable for the present state of confusion regarding the physiology of the gastro-intestinal canal.

Experimental data are available which indicate the myogenic nature of the rhythmic contractions of the stomach and intestine. The earlier work of Magnus, which has been quoted extensively in the literature, led him to conclude that these contractions are neurogenic. However, when he ('05) discovered that physostigmin brings out rhythmic contractions in completely denervated pieces of the intestinal musculature, he had to admit the myogenic nature of such contractions. Gunn and Underhill ('14) repeated this work of Magnus, using more refined technique, and obtained similar results. More recently, Alvarez and Mahoney ('22) presented further evidence which seems to indicate clearly the myogenic nature of the rhythmic contractions of the intestine.

If we may regard the rhythmic contractions of the stomach and intestine as myogenic, the problems regarding the activities of these organs become less baffling. The persistence of the rhythmic contractions following the administration of nicotin presents no further difficulty. Neither does it indicate that these contractions are not normally influenced by the enteric nervous mechanism. Some experimental evidence bearing on this point is already available. It is hoped that the rôle of the enteric



plexuses in the control of the rhythmic contractions of the stomach and intestine may be considered in the light of further experimental data in a later paper. On the other hand, the fact that the peristaltic contractions in the intestine, which must be regarded as neurogenic, are inhibited following the administration of nicotin, is in full accord with the discovery of synapses in the myenteric and submucous plexuses as set forth in this paper. Furthermore, all reflexes which, according to experimental evidence, are carried out within the walls of the alimentary canal are readily understood if we admit the occurrence of reflex arcs in the enteric plexuses. Therefore, the writer believes that available experimental data fully corroborate the anatomical findings set forth in this paper.

In an effort to illustrate the possible arcs mediating gastric and intestinal reflexes, the writer presented, in the earlier paper referred to above, a generalized scheme which includes afferent neurons in the myenteric plexus which send their axons centralward in the extrinsic nerves. The present study affords no evidence of such neurons. Experimental data also indicate that all gastric and intestinal reflexes which involve extrinsic nerves are carried out through the visceral afferent and efferent components of the cerebrospinal nerves. As set forth in the present paper, synapses in both the myenteric and submucous plexuses which involve two neurons located in the same ganglion suggest the occurrence of reflex arcs which involve only one or the other of these plexuses. Synapses which involve neurons in the myenteric plexus and fibers coursing in commissures which connect the latter with the submucous plexus indicate the occurrence of reflex arcs which involve both plexuses. The afferent limbs of these local reflex arcs have not been traced without interruption. Doubtless, the fibers which terminate in the gastric and intestinal epithelium are primarily the dendrites of neurons in the submucous plexus. Possibly, afferent fibers which arise in the myenteric plexus also reach the gastric and intestinal epithelium. However, the myenteric reflexes which are stimulated by distention of a segment of the gastro-intestinal canal suggest that the afferent limbs of the myenteric reflex arcs commonly terminate within the musculature.

## SUMMARY

The neurons in the enteric plexuses vary greatly in size and form and in the number, length, and distribution of their dendrites. They can probably not be classified according to two distinct morphological types. Both neurons with short, frequently branching dendrites and neurons with fewer long, infrequently branching dendrites occur in the myenteric plexus. Neurons with few relatively long dendrites preponderate in the submucous plexus.

Preganglionic fibers and fibers which arise in the submucous plexus make synapse with neurons in the myenteric plexus. Synapses involving two neurons in the same ganglion and synapses involving two neurons in separate ganglia in the same plexus occur both in the myenteric and the submucous plexus. Two neurons within the enteric plexuses which sustain a synaptic relationship to each other constitute a partial or complete local reflex arc.

The present study affords no evidence of the occurrence of nerve nets in the walls of the digestive tube.

Fibers which arise primarily in the submucous plexus terminate in the muscularis mucosae, among the glands in the mucosa, and in the gastric and intestinal epithelium. The fibers which terminate in the epithelium are probably the dendrites of neurons in the submucous plexus.

The occurrence of reflex arcs which comprise only neurons either in the myenteric or the submucous plexus as well as those which comprise neurons in both plexuses is indicated by the anatomical findings. Consequently, a structural basis is afforded for the rational interpretation of all the usual activities of the stomach and intestine except the rhythmic contractions. Attention is called to the work of Magnus, Gunn and Underhill, and Alvarez and Mahoney which indicates the myogenic nature of these contractions.

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Resumen por el autor, E. A. Boyden.

El desarrollo temprano de la cloaca en los embriones del avestruz, con especial mención de la reducción del intestino caudal.

El presente trabajo es el primero de una serie de artículos sobre la embriología del avestruz. En él se sigue el desarrollo de la cloaca hasta el estado de 18 mm.—esto es, hasta la aparición de la bolsa de Fabricio. Su interés principal está en la explicación que ofrece de la fenestra cloacal—una estructura descrita recientemente por el autor en los embriones de pato, faisán y gallina. El trabajo se ocupa también del seno urodeal, una repetición embrionaria de la vejiga dorsal de los saurios, el cual deriva, en el avestruz, del extremo proximal del intestino caudal. Dentro del periodo estudiado no existe oclusión del recto, pero el colon exhibe asas que le distinguen del de la mayor parte de las otras aves. En general, la cloaca del avestruz se parece más a la cloaca de los embriones jóvenes de reptiles que a la de cualquier otra ave estudiada hasta el presente.

Translation by José F. Nonidez  
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## THE EARLY DEVELOPMENT OF THE CLOACA IN OSTRICH EMBRYOS, WITH SPECIAL REFERENCE TO THE REDUCTION OF THE CAUDAL INTESTINE

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ONE PLATE (EIGHT FIGURES)

The cloaca of the ostrich presents two characteristics of unusual interest, the enormous size of the male copulatory organ and the persistence, throughout adult life, of an undiminished cloacal bursa (bursa Fabricii). As early as 1836, the first of these peculiarities attracted the attention of Johannes Müller, who described two types of erectile organs found in ratite birds and appended to this account a discussion of the developing form of copulatory organs in vertebrates in general. The second feature attained significance, somewhat later, through the investigations of Forbes, Wenckebach, and Gadow. These men demonstrated not only the persistence of the bursa in adult Ratitae, in contrast to its early atrophy in the young of carinate birds, but also its great size and the peculiar function it subserves as a urinary bladder.

Since the bursa of the ostrich differs so markedly from the corresponding organ in other groups, it occurred to me that a careful study of its development and histogenesis might throw some light on the significance of the bursa in flying birds, in which forms it has been variously interpreted as a lymphoid structure, a gland of internal secretion, a vestigial organ of obscure antecedents, etc. An opportunity to secure a graded series of embryos for this purpose was provided unexpectedly by a trip to California. It is a great pleasure, in this connection, to acknowledge the hearty cooperation of the manager and attendants of the Cawston Ostrich Farm at South Pasadena and the courtesy of Prof. A. W. Meyer, of Stanford University, who provided the reagents used in collecting the embryos.

The present paper is in the nature of a preliminary report and is restricted to a consideration of the early development of the cloaca, up to the stage ending with the appearance of the bursa of Fabricius. Its chief interest lies in the bearing which it has on the cloacal fenestra, a structure recently discovered by the author in embryos of the common fowl, duck, and pheasant.<sup>1</sup>

This fenestra was described as a temporary foramen, caused by the disintegration and subsequent removal of that portion of the cloacal wall subjacent to the dorsal aorta. It was held to be of special interest not merely because it furnished the only instance, in the differentiation of a hollow organ, in which a gap occurs in the epithelial wall as a normal and constant feature of development, but also because it provided landmarks which established for the first time the exact point of origin of the bursa of Fabricius.

The phenomenon was thought to be intimately associated with the retrograde process causing the atrophy of the caudal intestine, since the area of disintegration spread posteriorly into the caudal intestine soon after it appeared on the flanks of the cloaca (see vertically lined area in figure 4). This interpretation has been greatly strengthened by the series of ostrich embryos presented in figures 1 and 2 and 5 to 8, as well as by comparison with the development of the caudal intestine in turtle embryos.

To make the comparisons clear it is necessary to review the initial stage of fenestra formation indicated in figure 4. This is a reconstruction of a 41-somite chick embryo in which the ends of the Wolffian ducts are about to fuse with the cloaca. The caudal intestine is patent and its cavity extends from cloaca to tail-bud. The angle subtended by the cloaca and the caudal intestine (see area marked by crosses) contains a dense mass of undifferentiated tissue which, like the tail-bud, is a persistence of the primitive streak of earlier stages.<sup>2</sup> The epithelium

<sup>1</sup> The development of the cloaca in birds, with special reference to the origin of the bursa of Fabricius, the formation of a urodaeal sinus, and the regular occurrence of a cloacal fenestra. *Am. Jour. Anat.*, vol. 30, no. 2, March, 1922.

<sup>2</sup> The exact interpretation of this tissue is subject to reservation and awaits further investigation. The author has followed Gasser in deriving this from the primitive streak, with which it is admittedly continuous from its earliest appearance.



bordering this mass (that is, the inner curvature of the caudal intestine) and the wall of the cloaca adjacent to the anal plate are not fully differentiated from the primitive streak. The vertical lines in the figure indicate paired epithelial areas on the flanks of the cloaca which are about to disintegrate. The subsequent removal of these and adjoining areas by phagocytosis severs the caudal intestine from the cloaca, and exposes the contents of the latter to the mesenchyma through a broad fenestra. (For further details see publication referred to on page 212.) In the duck the cavity of the caudal intestine is occluded at a distance from the cloaca (cf. *x* in fig. 3), but does not rupture before the fenestra develops, whereas in the tern the tube becomes solid and ruptures at the point of occlusion, without the occurrence or intervention of a fenestra.

It is now appropriate to consider the reduction of the caudal intestine in turtle embryos. Figure 3 is a reconstruction of a 45-somite *Chrysemys* embryo slightly older, relatively, than the chick shown in figure 4. The caudal intestine has ruptured at *x* and its cavity on each side of the break is occluded. The primitive-streak mass has been reduced to a thin plate appended to the inner curvature of the caudal intestine. The most interesting feature is the method by which the cloacal end of the tube undergoes reduction. The vertically lined area in the figure marks the position of an external groove which corresponds to a longitudinal fusion of the lateral walls inside and a consequent obliteration of the cavity at this level. The effect of this fusion is two-fold. It has reduced the broad opening of the caudal intestine to a narrow passage in the lower segment of the cloaca and it has shifted its outlet forward as far as the level of the Wolfian ducts. The continuation of this process in later stages reduces even the lower segment to a solid plate of disintegrating tissue. The grooved area in figure 3 is undergoing phagocytosis and corresponds in position to the disintegrating area in the walls of the chick, which there becomes modified into a cloacal fenestra. And it accomplished the same result, namely, the obliteration of the proximal portion of the caudal intestine.

A third method of reducing the caudal intestine is exhibited by ostrich embryos. The first stage is shown in figure 1, of an embryo slightly older than the chick, but somewhat younger than the turtle embryo just described. The primitive-streak tissue has become separated from the anal wall of the cloaca, but still forms the inner curvature of the caudal intestine. As in the turtle, this tissue has been reduced to a thin plate one or two cells thick. It may be distinguished from the mesenchyma of the tail by its deeper stain and its homogeneous appearance. The once continuous lumen of the caudal intestine has been broken up into isolated cavities by the irregular adhesion of its walls, this process taking place in such a way as to give the cavities that remain a wave-like contour.

In the next stage, figure 2, the lumen of the caudal intestine is entirely obliterated and forms, with the primitive streak, a ribbon-like band of tissue running through the core of the tail. In the youngest portion of the caudal intestine, near the end of the tail, the lumen still persists and both caudal intestine and primitive streak, as in figure 1, merge with the tail-bud.

In the third stage, represented by figures 5 and 6, the caudal intestine has ruptured from the cloaca, and its distal end, except that portion in contact with the tail-bud, has disappeared. The proximal end is still attached to the cloaca, but its outlet has been shifted as far forward as the Wolffian duct. Whether this takes place by such a progressive adhesion of lateral walls as occurs in the turtle, or is accompanied by the breaking through of an adhesion, such as occurs at the point indicated by an asterisk in figure 5, is uncertain, since two of the available embryos of this age were like figure 5 and two were like figure 6. While the first type may be anomalous and may represent merely a delayed fusion of the walls in the region behind the break, it is quite possible that figure 5 is the step which normally precedes the stage shown in figure 6.

There are thus presented, in the Sauropsida, three modifications of a method by which the cloacal end of the caudal intestine is reduced. In turtles the projecting stump of the tube is constricted off by a progressive adhesion of the lateral walls of the

cloaca. In the ostrich, in at least two cases, the constriction breaks through in the middle after the manner in which the semicircular canals of the ear are cut out of the otocyst. In the duck and gallinaceous birds the corresponding areas of the cloacal wall disintegrate before adhering, thereby producing a temporary lesion known as the cloacal fenestra. It is significant that the most conspicuous of these methods, that found in the chick, is associated with the persistence of the greatest amount of undifferentiated primitive streak and that in birds like the tern, in which none of the undifferentiated tissue remains, the caudal intestine ruptures at the proximal end and is absorbed into the cloaca as uneventfully as in mammals.

The ultimate disposition of the cloacal end of the caudal intestine in the ostrich is very surprising. A comparison of figures 6, 7, and 8 shows that the blunt end of the tube becomes converted into a permanent diverticulum (diverticulum 'c' of figure 8) which corresponds in position to the urodaeal sinus of the chick embryo, a structure which was interpreted to be a repetition of the dorsal bladder found in snakes and lizards. In the domestic fowl this diverticulum arose as a new outpocketing of the dorsal wall between the openings of the two Wolffian ducts, soon after the fenestra had severed the outlet of the caudal intestine from the cloaca. Its mode of origin in the ostrich and tern embryos suggests that the urodaeal sinus of all birds is primarily a derivative of the cloacal end of the caudal intestine.

The ostrich cloaca also suggests a possible explanation for the two accessory diverticula in the chick, defined as diverticulum 'a' and 'b.' When these were first seen it was thought that they were irregularities left at either end of the fenestra by that destructive process, but a study of the ostrich series shows that diverticulum 'a' is a constant feature in a bird which never develops a cloacal fenestra. It appears in stages represented by figures 5 to 7, and either becomes incorporated in the bursa or disappears before the latter is formed. In the chick it happens to lie at the caudal boundary of the fenestra, and this incidental relation may explain its larger size in that embryo. But in both birds it is a temporary structure, and the only definite thing

about its position is that it arises at the point where the primitive streak originally joined the cloaca.

Diverticulum 'b,' on the other hand, is not found in the ostrich, and only occasionally in the chick, where it appears at the cephalic end of the fenestra and always adjacent to the urodaeal sinus (figs. 24, 28, 30, and 32 of the previous paper). This double relation, in view of the origin of the sinus in the ostrich, suggests that diverticulum 'b' in the chick may represent a remainder of the cloacal end of the caudal intestine left by the formation of an incomplete fenestra. This, in turn, raises the question as to whether the urodaeal sinus of the ostrich may not be of double origin, arising chiefly from the remainder of the caudal intestine, but also in part from a possible, independent outpocketing of the cloacal wall comparable to diverticulum 'c' of young chick embryos. In figures 5 and 7 such a diverticulum is barely suggested in the region between the caudal intestine and the rectum. The issue raised, however, is a fine one and does not jeopardize the statement that the bulk of the urodaeal sinus is derived from the cloacal end of the caudal intestine.

Another interesting feature of this region in the ostrich is the coiling of the large intestine, the beginning of which process can be seen in figures 7 and 8. In most other birds the large intestine remains a short straight segment of the gut, leading from the intestinal caeca to the cloaca. But in *Struthio*, as Owen pointed out long ago, the large intestine is much coiled and twice as long as the small intestine. In view of this fact, Owen's use of the term rectum to include the whole length of the large intestine in birds seems ill advised; and it is to be questioned whether Gadow's statement that the colon is present only in *Struthio* can be justified.

The unusual size of the intra-embryonic portion of the allantois also merits attention. This structure in the chick was described as an exact duplication of the dilated area in reptile embryos which develops into the ventral bladder of the adult. In the ostrich it is even more conspicuous than in the chick, having a recurrent lobe (fig. 8, *all. bl.*) which fills up the posterior end of the body cavity. But in both species it disappears before hatching.

Two other respects in which the ostrich differs from the chick are the failure of the rectum to become occluded before the 18-mm. stage is reached and the modification of the method by which the urodaeal membrane is formed. The combination of these two processes in the chick restricts the cavity of the cloaca to a narrow channel connecting the Wolffian ducts with the allantois, the alleged purpose of which, as suggested by Parker, is "to prevent the escape of the excretion either into the intestine or into the amniotic cavity, where it might prove injurious to the embryo." Although the occlusion of both regions is completed by the 15-mm. stage in the chick, there is no narrowing of the lumen in the rectum of an 18-mm. ostrich, while the urodaeal membrane is barely indicated by the approximation of the flanks of the cloaca (dotted area, fig. 8). Yet in this same embryo the metanephros is as far along as in a 15-mm. chick. It is not improbable that the examination of older embryos will show that there is no solid stage to the large intestine in the ostrich.

The peculiarity of the urodaeal membrane in the ostrich lies in the order of its formation. In the chick the lateral walls of the cloaca first meet in the region of the anal plate, then along the caudal margin of the cloaca. From these two places the area of fusion spreads anteriorly toward the allantois and rectum (cf. plate 3 of article referred to on page 212), until all that portion of the cavity has become obliterated and transformed into a flat membrane. In the ostrich (dotted area, figs. 6 to 8) it begins at the anal plate and extends to that part of the caudal wall adjoining the urodaeal sinus, thereby leaving a deep pocket adjacent to the anal plate. This has an important bearing on the origin of the bursa, because in all other birds studied the bursa originates from a proliferation of that portion of the caudal margin nearest the anal plate. But in figure 8 the primordium of the bursa (indicated by three big vacuoles in the lateral wall of the cloaca as well as by the thickened caudal margin) is located as near to the urodaeal sinus as it is to the anal plate. It is quite probable that the undotted area near the anal plate is merely the last portion of the cavity to be reduced, but the reversal of order

is significant as being the first difference noted between the development of the bursa in carinate and ratite birds. It would seem to indicate that the bursa of the ostrich will occupy all the margin of the cloaca between the urodaeal sinus and the anal plate, thereby explaining the unusually wide orifice of the bursa in the adult bird which, it is said, partially houses the retracted penis.

The early development of the cloaca in the ostrich thus offers many interesting points of comparison with the cloaca of other bird embryos. And, in general, it exhibits a distinctly more reptilian character than the cloaca of carinate embryos—in the method by which the caudal intestine is reduced, in the position of the Wolffian ducts, in the size of the allantoic bladder, in the very thin anal plate, in the elongation of the large intestine, and in length of tail and number of caudal somites. It is such primitive characters as these which arouse the hope that further study of this series of embryos will contribute much to an understanding of the significance of the bursa of Fabricius and the origin, in general, of avian from reptilian structure.

PLATE

## PLATE 1

### EXPLANATION OF FIGURES

Graphic reconstruction of sauropsidan embryos drawn to the same scale.  $\times 35$ . This plate should be compared with plates on pages 167 and 199, *Am. Jour. Anat.*, vol. 30, 1922. Dash lines indicate cavities; dotted lines, vacuoles or somites; dotted areas, regions in which opposite walls of the cloaca are nearly in contact; areas with crosses, primitive-streak remainders; vertically lined areas, areas of disintegration. *a* represents a constant, though temporary diverticulum homologous with diverticulum *a* of chick embryos. *c* represents a diverticulum which regularly forms the medial component of the urodaeal sinus: in ostrich embryos it is derived from the proximal end of the caudal intestine.

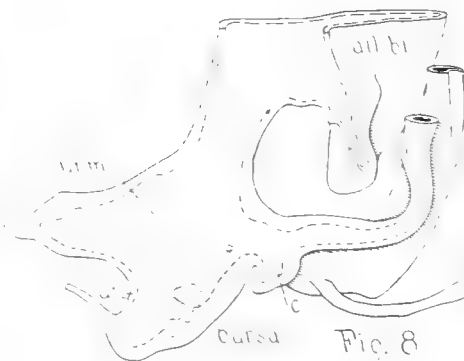
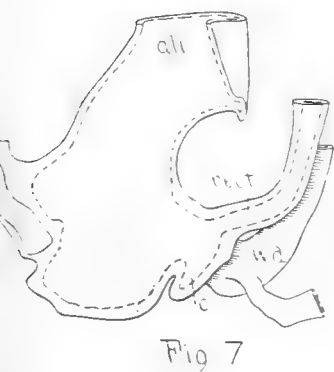
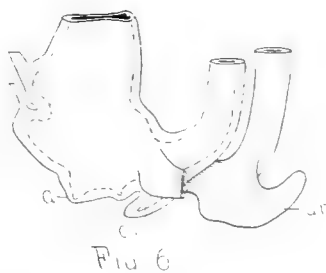
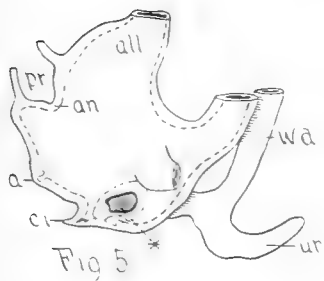
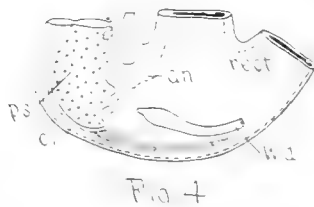
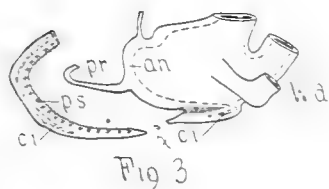
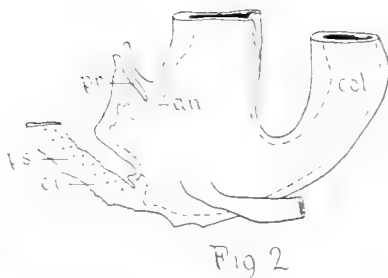
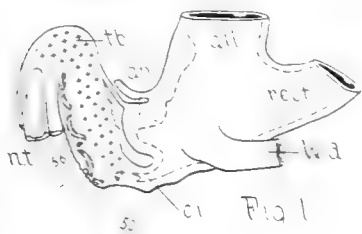
- 1 Ostrich embryo (*Struthio australis*<sup>1</sup>), H.E.C. 2234. 56 somites.
- 2 Ostrich embryo, H.E.C. 2235. 7 mm.
- 3 Turtle embryo (*Chrysemys marginata*), H.E.C. 1067. 6 mm. (after R. F. Shaner).
- 4 Chick embryo, H.E.C. 2071. 41 somites. 3 days, 18 hours.
- 5 Ostrich embryo, H.E.C. 2239. 10.2 mm.
- 6 Ostrich embryo, H.E.C. 2240. 10 mm.
- 7 Ostrich embryo, H.E.C. 2243. 13.5 mm.
- 8 Ostrich embryo, H.E.C. 2245. 18 mm.

### ABBREVIATIONS

<i>a</i> , diverticulum 'a'	<i>pelv.</i> , pelvis of kidney
<i>all.</i> , allantois	<i>pr.</i> , proctodaeum
<i>all.bl.</i> , allantoic bladder	<i>p.s.</i> , primitive-streak remainder
<i>an.</i> , anal plate	<i>rect.</i> , rectum
*(asterisk), area occupied by mesenchyma	<i>umb.</i> , constriction of pelvis caused by umbilical artery
<i>bursa.</i> , bursa cloacae (Fabricii)	<i>ur.</i> , ureter
<i>c.</i> , diverticulum 'c' (urodaeal sinus)	<i>ur.m.</i> , urodaeal membrane
<i>c.i.</i> , caudal intestine	<i>W.d.</i> , Wolffian duct
<i>col.</i> , large intestine	<i>x.</i> , rupture of the caudal intestine
<i>n.t.</i> , neural tube	

<sup>1</sup> All the ostriches figured on this plate are probably *Struthio australis*, the South African species. There is a slight possibility that the specimens contain a strain of the big Nubian ostrich, *Struthio camelus*.





Resumen por los autores, P. N. Johnstone y F. H. Wakefield.

Sobre el carácter de las fibras de Purkinje en varias  
regiones del fascículo atrio-ventricular.

La presente investigación se ocupa de la morfología de las fibras de Purkinje en varias regiones del fascículo atrio-ventricular. El método empleado consiste en cortar en serie bloques de tejido tomados de las regiones objeto de estudio, reconstruyendo las fibras de Purkinje. Con este objeto los autores han empleado el método de Born mediante placas de cera y el método consistente en la superposición y combinación de cortes seriados dibujados en papel. Los autores han llegado a las siguientes conclusiones mediante el estudio de otros cortes y las reconstrucciones: 1) Las fibras de Purkinje del nodo atrio-ventricular, conforme ha demostrado Tawara, son mucho más pequeñas y se ramifican y anastomosan más extensamente, que en cualquier otra región del fascículo atrio-ventricular. 2) Las fibras de Purkinje del tronco y de los miembros derecho e izquierdo del fascículo son muy semejantes; las del tronco son más sinuosas. En corte transverso presentan una forma algo cilíndrica. Aunque son mayores que las del nodo, parecen mucho más pequeñas que en las expansiones terminales. Se ramifican y anastomosan menos, que las fibras del nodo, pero más que las de las expansiones terminales. 3) Las fibras de Purkinje de las expansiones terminales son polimórficas. Hay dos tipos extremos: el de forma de bastón y el foliáceo. En nuestra reconstrucción de las fibras mencionadas del músculo papilar posterior del ventrículo izquierdo la fibra foliácea está algo fenestrada. 4) La acumulación de tejido de Purkinje en las expansiones terminales es mucho mayor que en el tronco y miembros del fascículo. 5) Los autores no han encontrado porciones aisladas del tejido de Purkinje.

Translation by José F. Nonidez  
Cornell Medical College, New York.

## ON THE CHARACTER OF THE PURKINJE FIBERS IN VARIOUS REGIONS OF THE ATRIO- VENTRICULAR BUNDLE

PAUL N. JOHNSTONE AND FRANK H. WAKEFIELD

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### SIX FIGURES

Purkinje, in 1845, first called attention to the presence in the subendocardial layer of the sheep's heart of a system of fibers that are of a different character from heart muscle. These fibers are found in the ventricles ramifying over their surfaces. Numerous writers have since pointed out, along with Purkinje, that they are to be found in the hearts of many other animals including the pig, calf, goat, horse, dog, cat, rat, mouse, goose, hen, dove, and others. They are usually situated immediately under the endocardium, but are at some places to be found in the myocardium. Hoffman has given evidence that they are to be found in the pericardium. If the left ventricle of a fresh sheep's heart is opened the fibers that were first described by Purkinje will appear as glistening thread-like strands. They are present in almost all the regions of the ventricle, but are noticeably absent in certain regions, including that portion of the ventricular septum immediately beneath the septal cusp of the aortic valve. They are to be found in great abundance in the regions of the trabeculae carneae where they seem to pass as single strands from trabecula to trabecula. They are seen in considerable quantities at the bases of the papillary muscles and in some instances find their way almost to the apices. As seen macroscopically on the inner surface of the ventricle, they are found to branch and anastomose quite extensively, forming a most intricate network.

W. His, Jr., in 1893, discovered a bundle of fibers, which he thought to be muscle, connecting the right atrium with the

ventricles. This bundle of fibers known as the atrioventricular bundle, so named by His, arises in the right atrium from a node known as the atrioventricular node. This node was discovered by Tawara and is described in his monograph. It lies beneath the coronary sinus of the right atrium and approaches the atrial septum more nearly than does the coronary sinus. The atrioventricular bundle continuing from the node travels in the general direction of the ventricular septum. Upon reaching the septum it divides into a right and a left limb. In the calf's heart the right limb is at first subendocardial, but as the septum thickens it becomes imbedded to a depth of several millimeters in the heart muscle of the ventricular septum. The left limb is found immediately beneath the endocardium. After traversing a short distance, the left limb bifurcates into an anterior and a posterior part, while the right limb continues undivided. In his investigations Tawara concluded that the fibres which were described by Purkinje were merely continuations of the atrioventricular bundle. Lydia DeWitt conclusively proved that this was the case by dissecting the entire structure and then modeling it to scale.

All of the fibers of the sinoventricular system—though differing greatly in size, shape, and arrangement in its various regions—because they are composed of a related if not an identical tissue will hereafter in this report be called Purkinje fibers. The tissue composing the Purkinje fibers will be termed Purkinje tissue. Tawara in his monograph has divided the atrioventricular bundle into two main parts, the atrial part and the ventricular part. He subdivides the atrial part into the node and the trunk, and the ventricular part into the upper undivided part and the terminal expansions, or the Purkinje fibers. In this report we shall employ Tawara's classification in the main. We shall not use the terms 'terminal expansions' and 'Purkinje fibers' synonymously. For the purpose of clearness, we have outlined the nomenclature that is employed in this report (fig. 1).

Our method of studying the Purkinje fibers in the various regions of the atrioventricular bundle consisted essentially of making serial sections of the fibers from the regions to be studied

and of reconstructing them. All of the sections were taken from one calf heart. Akey gives a diagrammatic representation of the atrioventricular bundle representing the regions from which the reconstructions were made. The heart was procured

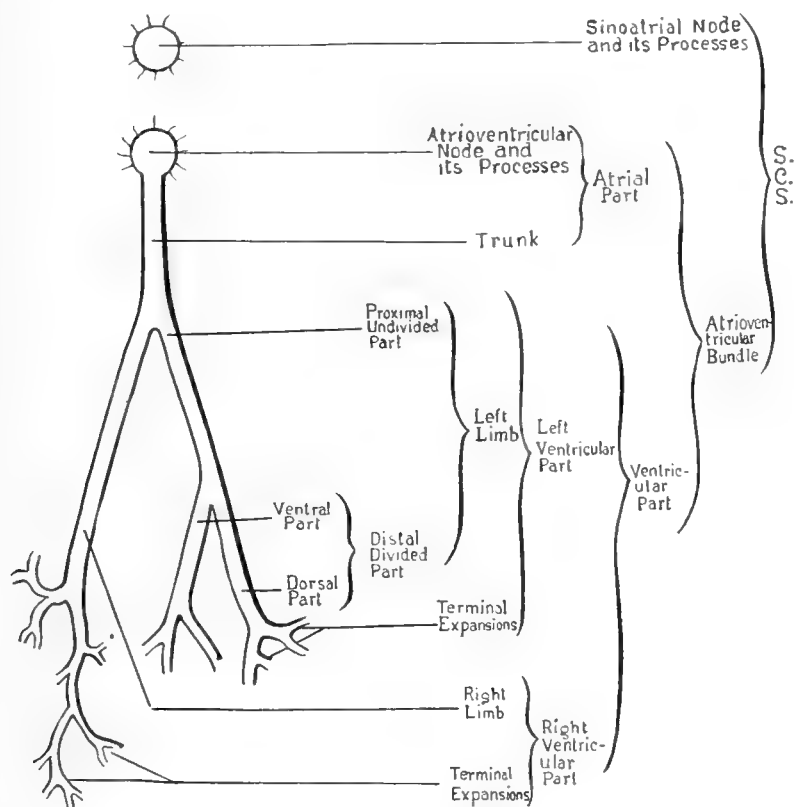
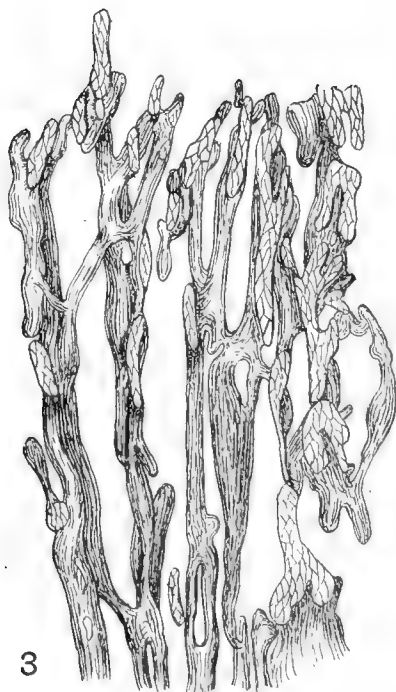
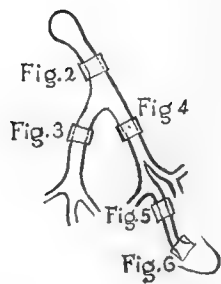


Fig. 1 An outline of the nomenclature used in this paper. Purkinje fibers: all of the fibers of the sinoventricular conducting system that are composed of Purkinje tissue. S. C. S. = sinoventricular conducting system.

immediately after the animal was killed. It was carried to the laboratory and there opened in the following manner: An incision was made along the marginal surface of the left ventricle and atrium. With the scissors the aorta was opened along a line drawn through the left sinus of Valsalva and the apex of the left



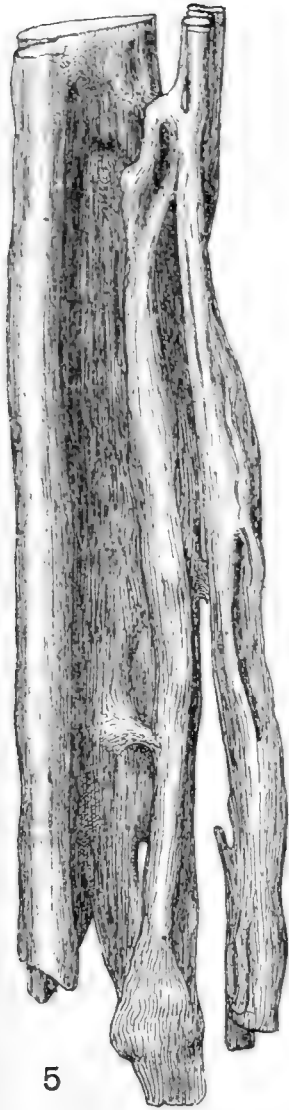
2



3



4



5



6

Figs. 2 to 6 Drawings of reconstructions of various parts of the atrio-ventricular bundle of the calf's heart. All five were taken from the same heart, fixed in Carnoy's fluid, imbedded in paraffin, sectioned at  $25\ \mu$ , and stained with hematoxylin and eosin. All are reconstructed at the same magnification of 73.2 diameters, reduced one-third in reproduction, to a magnification of 48.8 diameters. Only Purkinje tissue is reconstructed.

The reconstructions shown in figures 2 to 4 were made by superimposing and combining serial sections on paper. Those shown in figures 5 and 6 were made by the Born wax-plate method. Figure 2 is from the trunk. Figure 3 is from the right limb. Figure 4 is from the left limb. Figure 5 is from a moderator band of the left ventricle. Figure 6 is from the posterior papillary muscle of the left ventricle.

ventricle. The anterior surfaces of the pulmonary artery, the conus arteriosus, and the right ventricle were cut through with the scissors. When the apex of the ventricle was reached the cut was turned directly along the marginal surface of the right ventricle, through the atrioventricular junction and along the marginal surface of the right atrium. This exposed the atrioventricular bundle without damaging it. Blocks of tissue were taken from it in the following regions: 1) the trunk, 2) the right limb, 3) the proximal undivided part of the left limb, 4) a moderator band of the left ventricle, 5) the posterior papillary muscle of the left ventricle. The blocks were all made large where possible so as to avoid any possible distortion of the Purkinje fibers due to curling. They were then fixed in Carnoy's solution, imbedded in paraffin, and sectioned at a thickness of 25  $\mu$ . In all cases the Purkinje fibers were cut longitudinally. The sections were stained with hematoxylin and eosin and were mounted in balsam-damar.

No difficulty was encountered in making the reconstructions of the Purkinje fibers of the moderator band and of the papillary muscle by the Born wax-plate method. The Purkinje fibers of the trunk, the right limb, and the left limb were reconstructed by the method of superimposing and combining successive sections on paper. It was found that they were too small at the magnification planned to admit of accurate results by the wax-reconstruction method. The reconstructions were all done at a magnification of 73.2 diameters.

In his monograph Tawara has shown very good illustrations of the Purkinje fibers of the atrioventricular node which he described. Our sections have only confirmed the findings of Tawara, that the Purkinje fibers of the atrioventricular node are very much smaller and very much more tortuous than are the fibers of the trunk. Our reconstructions of the Purkinje fibers of the trunk show that they have a gnarled arrangement and that they branch and anastomose quite extensively (fig. 2). The Purkinje fibers of the right and left limb are virtually identical and are less gnarled than are those of the trunk (figs. 3 and 4). The size of the Purkinje fibers of the two limbs in com-



parison with those of the trunk is about the same. Individual Purkinje fibers in either of these three regions vary in size. In cross-sections made from other hearts the Purkinje fibers of the trunk and of the limbs appear to be somewhat cylindrical in shape.

The reconstruction of the Purkinje fibers of the moderator band shows the fibers to be very much larger, and that very much less branching and anastomosing takes place than in either the limbs or the trunk (fig. 5). Moreover, the Purkinje fibers seem to be of two kinds in this reconstruction, one being more cylindrical in shape and the other of the character of a sheet. The Purkinje fibers in the reconstruction of the moderator band that are more rod-like in shape are many times as large as the fibers in the limbs and the trunk. The Purkinje fiber having the character of a sheet in this reconstruction is very broad. It is nearly as broad as all the other Purkinje fibers of the moderator band. It is not fenestrated. One of the more rod-like Purkinje fibers is hollow throughout a part of its course. This tubular formation is finally terminated at a mouth which opens in the side of the fiber.

The Purkinje fibers which were reconstructed from the papillary muscle show a broad sheet-like fiber which is somewhat fenestrated, along with several instances of rod-like fibers (fig. 6). These are apparently continuations of the corresponding types seen in the moderator band. Our reconstruction, along with a study of sections from other hearts, leads us to believe that these fibers are polymorphous. These shapes we believe to be bounded by the rod-like and the sheet-like fibers as the two extremes. The sheet-like fiber of the papillary muscle is the largest fiber in our reconstructions. The increase in the size of the Purkinje fibers in the terminal expansions is typical, as examination of sections from other hearts shows this characteristic. As isolated portions of Purkinje tissue were not found in any of the reconstructions, it is reasonable to suppose that they do not exist.

## SUMMARY

In this investigation the Purkinje fibers in various regions of the atrioventricular bundle were studied by reconstruction methods. It has been found that—

1. The Purkinje fibers of the atrioventricular node, as was shown by Tawara, are very much smaller and branch and anastomose more extensively than in any other region of the atrioventricular bundle.

2. The Purkinje fibers of the trunk and of the right and left limbs are very similar, the fibers of the trunk being more gnarled. In cross-section they seem to have a somewhat cylindrical shape. Though they are larger than in the atrioventricular node they are much smaller than in the terminal expansions. They branch and anastomose less than the Purkinje fibers in the atrioventricular node but more than the Purkinje fibers in the terminal expansions.

3. The Purkinje fibers of the terminal expansions are polymorphous. There are two extreme types, the rod-like and the sheet-like. In the reconstruction of the Purkinje fibers of the moderator band of the left ventricle the sheet-like fiber is not fenestrated, but in the reconstruction of the Purkinje fibers of the posterior papillary muscle of the left ventricle the sheet-like fiber is somewhat fenestrated.

4. The accumulation of Purkinje tissue in the terminal expansions is far greater than in the trunk or in the limbs.

5. Isolated portions of Purkinje tissue were not found.

In conclusion, we wish to express our appreciation to Dr. E. R. Clark for the assistance that he has rendered in the carrying out of this investigation. We wish also to acknowledge the services of Mr. G. T. Kline, who made the final drawings of the illustrations.

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Resumen por el autor, John S. Latta.

La interpretación de los llamados centros germinales del tejido linfático del bazo.

Desde la publicación de los resultados de la investigación de Flemming ('85) en los cuales dicho autor concluye que las áreas centrales más claras de los nódulos linfáticos densos son centros de proliferación, o "centros germinales," los investigadores que han estudiado el problema del tejido linfático han estado acordes invariablemente con este concepto de su significación. En un estudio reciente ('21) de estas estructuras en el tejido tonsilar intestinal, no he encontrado prueba alguna de la actividad proliferativa de estas áreas, sino que he llegado más bien a la conclusión de que representan centros de degeneración. Un cuidadoso estudio histogenético ha sido llevado a cabo por el autor en los centros de los nódulos linfáticos esplénicos. En estas regiones no he podido hallar prueba alguna de actividad proliferativa o transformativa. El aspecto más claro de estos centros, cuando existen, se debe a una menor densidad de los linfocitos libres en este sitio, asociada con mayor prominencia del tejido reticular. El autor ha encontrado estados de la degeneración de los pequeños linfocitos y hemoblastos linfoides, los cuales se deben, sin duda alguna, a la disminución de la nutrición de los centros. El autor indica, por consiguiente, que el término "centro germinal" no describe las condiciones existentes en estos puntos, y que deben considerarse más bien como centros en los cuales tienen lugar cambios degenerativos.

Translation by José F. Nonidez  
Cornell Medical College, New York.

## THE INTERPRETATION OF THE SO-CALLED GERMINAL CENTERS IN THE LYMPHATIC TISSUE OF THE SPLEEN

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THREE FIGURES

In the histological examination of dense lymphatic tissue as found in the lymphatic nodes, the various tonsils, and in the spleen of mammalian forms, it is noted that often the central portions of the lymphatic nodules are of lighter appearance than their peripheral portions. This fact was first studied in detail by Flemming ('85), who designated these lighter central portions of nodules, 'Keimzentrum,' or germinal centers, for he considered these areas of the nodules as centers of proliferation of lymphocytes. Since that time investigators have invariably followed Flemming in his interpretation of these light colored areas. There has been, however, apparently some confusion as to exactly what is meant by the term, germinal center, for several authors have included in this term an entire lymphatic nodule (Baum u. Hille, '08; Bunting, '05), instead of their lighter colored central portions, as evidently intended by Flemming and most investigators following him.

As I have indicated in a previous paper ('21), in the study of the lymphatic tissue of the intestinal tonsils, it seemed that the term, germinal center, was non-descriptive of conditions existing in those nodules of the intestinal wall which contained these light colored central areas. Results of this study seemed to indicate that the so-called 'germinal center' was not a place of special lymphocytic formation and proliferation, but rather one of degenerative changes.

Whether these centers are proliferative or degenerative in character, their interpretation is very closely associated with the nodular blood supply (i.e., the question of nutrition). In this respect, the Malpighian bodies (lymphatic nodules) of the spleen are of special interest because of the fact that they here develop as an hypertrophy of portions of the lymphoidal arterial sheaths, thereby changing, to some extent, the usual vascular relationships of nodules in lymphatic tissue. Then, too, the histological appearance of splenic nodules which possess the so-called 'germinal centers' seems to differ somewhat from nodules in other varieties of lymphatic tissue. Therefore, it is thought, that some study of the splenic lymphatic tissue, with special reference to the presence of the lighter colored central areas, their relation to the vascular supply, and their histological structure and development, might aid in more clearly interpreting their significance.

Human spleens, collected from autopsies performed at the University Hospital, University of Nebraska, in which the causes of death were, in no apparent way, associated with the spleen, were used chiefly in this investigation. These were from individuals of ages varying from still-born infants, in which the splenic nodules were ill-defined and few in number, to adults of somewhat advanced age. This human material was supplemented by spleens of dogs, cats, guinea-pigs, and rabbits, which were used for comparative purposes.

The fixing fluid found to give the best results with the stains which followed, and hence that used most extensively, was Helly's fluid or Zenker-formalin, prepared by adding 10 per cent of formalin to Zenker's stock solution. Picro-aceto-formalin (Bouin's fluid) was also used to some extent with very good results.

All of the material was imbedded in paraffin, the sections cut from 6 to 8  $\mu$  thick, and portions mounted serially, to be sure that correct relationships between the nodules and the surrounding parts could be determined.

The most commonly used stains were the blood stains, Hastings-Nochts modification of the Romanowski blood stain, and a

$\frac{1}{2}$  per cent solution of alcoholic eosin in methyl alcohol, followed by an aqueous solution of methylene blue, made slightly alkaline with KOH (as used by Gage). A solution composed of equal parts of 1 per cent aqueous solutions of methyl green and pyronin gave good results (after Pappenheim). Finally Mallory's aniline-blue connective-tissue stain was used, mainly upon tissue which had been previously fixed in picro-aceto-formalin, to show clearly the extent and amount of the reticular tissue in its relation to the splenic nodules.

In 1854, Brücke, in a study of lymphatic tissue, noticed that occasionally the dense nodules of this tissue had lighter colored central areas. He said, "One sees occasionally in the cortical nodules and still more often in Peyer's patches a central lighter portion." His ('62) also had observed lighter areas in the center of lymphatic nodular masses, which he called 'Vacuolen.' Frey ('74) said, "In the large follicular masses are seen lighter colored translucent centers." These centers, he considered, were the true follicles, the darker periphery being something peculiar to these follicles.

Flemming ('85) made a more exhaustive study of lymphatic tissue than previous investigators. He used as objects of study mesenteric lymphatic nodes from the ox and rabbit, Peyer's patches from the caecum of rabbits, and human lingual tonsils. He observed these lighter central areas in all of these different tissues, and considered them to be of the same significance in the tonsils, lymphatic nodes, and the spleen. In the center were cells with a large amount of cytoplasm and large nuclei, while cells at the periphery were smaller and stained more deeply, thus giving a lighter appearance to the central portion. In the center mitotic figures were prominent and at the periphery were scarce. Consequently he called this lighter central area the 'germinal center.'

Subsequently, as has been before intimated, there has been some confusion in regard to the 'germinal center,' and to what the term should be applied. Leading text-books have invariably followed Flemming in their interpretation of this structure, but in the study of the results of investigators of problems relating

to lymphatic tissue, one finds that the term 'germinal center' is often used in an indefinite way. The use of the term by Bunting ('04) and Baum and Hille ('08) has already been referred to. Gulland ('94) also seems to have had a rather vague idea of the significance of this term. He says the germinal centers (in lymphatic tissue) are known as light circles with dark circumferences. Sabin ('05) considers the second part in the development of lymph nodes to be the formation of germ centers, which latter she described as condensations of cells where lymphocytes are actively proliferating (also Ribbert, '89; Richter, '02).

These descriptions seem unsatisfactory as concerning the so-called 'germinal centers,' but correspond rather with current conceptions of complete dense lymphatic nodules.

Czermack ('93) followed Flemming in believing the 'germ centers' of lymphatic nodules to be composed of cells which continually produced lymphocytes. These cells he called 'Keimzellen.'

Saxer ('96), in speaking of 'germ centers,' said that the presence of more numerous divisions of fixed cells in the germ centers than in reticular and areolar tissue in other places is understandable because of the increased physiological function and the importation of nutritive elements. Just what he included under the term 'Keimzentrum' could not be determined.

All recent investigators who have studied the 'germinal centers' of lymphatic tissue agree with Flemming in the general interpretation of their significance (Schridde, '07; Maximow, '09; Downey and Weidenreich, '12; Hartmann, '14; Thiel and Downey, '21; Nakahara and Murphy, '21). The lymphoblastic nature of cells in the 'germinal center' has been quite generally accepted. Some authors have followed Czermack in giving these cells the specific name of 'germinal center cells' (as Hartmann, '14). Thiel and Downey, in discussing the germinal centers of the spleen, considered these areas as regions of special activity of the reticular tissue, as manifested by the hypertrophy, increase in number, isolation, and transformation of its cells.



In addition to cells of a lymphoblastic character, there have been demonstrated in the light colored 'germinal centers' of lymphatic tissue in various places certain large phagocytic cells (those containing the 'tingible Körper' of Flemming), considered by some to be derived from cells of the reticular tissue (Weidenreich and Downey, '12; Hartmann, '14), and, as studied by myself in intestinal tonsillar tissue ('21), thought to be derived by further differentiation and degeneration of lymphoid hemoblasts.

As the special proliferative activity of the 'germinal centers' is accounted for by many as a result of increased physiological function and unusual possibilities of nutrition (Saxer, Thiel and Downey, et al.), and as my previous explanation of them as degenerative centers is based upon an insufficient blood supply at these points, the relation of the lymphatic nodules to the vascular supply was a question of special interest in the present investigation.

Although, as before stated, splenic tissue from the dog, cat, and other forms were studied for comparison with the structure of the human spleen, in all respects in which comparison was made there was little or no variation, so the conclusions arrived at in this paper are based upon the study of the human spleen.

It becomes very evident, when sections of the spleen from individuals of varying ages are studied, that arteries of the spleen very early (before birth) acquire lymphoidal sheaths (which, according to Danchakoff, Downey, and others, is preceded by a mesenchymatous sheath). At the time of birth, however, there is little distinction to be drawn between the red and the white pulp (lymphatic tissue). The lymphoidal arterial sheaths, however, seem to increase markedly in density, and, in young adults, are a very distinctive splenic structure. Lymphatic tissue was very abundant in all of the adult human spleens studied, more particularly in their central portions.

Malpighian bodies, or splenic lymphatic nodules, are not recognized until some time after the formation of the lymphoidal arterial sheaths. They are formed, according to Downey and Weidenreich ('12), by the rapid increase of the number of lymphocytes, derived from fixed cells of the reticulum, in certain

regions of the lymphoidal arterial sheaths. These regions of increased proliferative activity are supposedly at points where there has been formed a dense capillary network which is absent in other places in these sheaths (Thiel and Downey, '21). By the study of serial sections of spleens, in some cases of which the arteries had been injected with a carmine gelatin mass and others which had become highly congested with blood, it was demonstrable that the splenic nodule (Malpighian follicles) were always very well supplied with capillaries, which branches from the central artery, so that the first development of these nodules is undoubtedly due to an increase in nutrition and metabolic rate at these particular points.

The lighter colored central areas, which according to Thiel and Downey may also be peripherally located in the nodules, are not present in the nodules at first formed. In fact, the so-called 'germinal centers' are infrequently found at any time in splenic nodules as compared to nodules of the various tonsils and of the lymphatic nodes.

In splenic nodules, in which the lighter colored central areas are apparent, the accompanying artery is always seen to be extremely excentric to the nodular mass, and never in the lighter colored portion. Nodules which possess no lighter colored 'germinal center' are relatively smaller and the artery is usually located centrally in the nodule or nearly so. Neither is the so-called 'germinal center' a region of extraordinarily rich vascularity, as was found in the nodules at the time of their formation. In most cases no capillaries or blood could be demonstrated in these regions, the increase in size of the nodules and their increase in density at the periphery apparently inhibiting the vascular supply to the central portions. In some nodules the vascular supply was found to be very extensive throughout the entire nodule, as at the time of their formation, but these nodules never exhibited a 'germinal center.'

The histological appearance of these 'germinal centers' was first discussed by Flemming ('85), who found their lighter appearance to be due to the preponderance here of cells with large, vesicular nuclei, which were also relatively rich in cytoplasm

(lymphoid hemoblasts), so that the nuclei were farther separated from one another here than at the nodular periphery. This phenomenon was observed by later authors, and the large cells apparently peculiar to the areas were called by some the 'germinal center' cells (Czermack, '93; Hartmann, '14).

Thiel and Downey, in their study of the development of the mammalian spleen, found that the 'germinal centers' were areas in which the reticular tissue, particularly the reticular cells, were hypertrophied and were specially active in the reproduction and transformation into lymphocytes, thus disagreeing with Saxer ('96), who, in the study of the development of lymphatic nodes, said, "The presence of a fixed mother tissue for the production of lymphocytes is neither practically demonstrable nor a theoretical postulate."

In the present study of the structure of the so-called 'germinal centers' as they appear in lymphatic nodules of the human spleen, it was found that, in these locations, the lymphocytes were much less closely packed together than at the periphery of the nodules. The cells in these centers were surely not predominately of the lymphoid hemoblast (lymphoblast, germinal center cell) type, as found by Flemming, Czermack, Hartmann, et al., the proportion between the small lymphocytes and the lymphoid hemoblasts remaining the same as at the periphery. Fibers of the reticular tissue are possibly more prominent in the 'germinal centers' than elsewhere in the nodules; this fact being due, it is thought, rather to the lesser density of the free lymphocytes and an increase of space between them, than to an hypertrophied condition of the reticulum. Nuclei of reticular cells were seen in the loose central portions, but not in unusual numbers, certainly not to a great enough extent to warrant a statement that the reticular cells are here specially active as a mother tissue productive of lymphocytes. It would seem that such prominence as the reticular tissue and cells may have in the so-called 'germinal centers' can be accounted for solely by the lesser number and density of the free lymphocytes in these places.

Evidences of proliferation of cells as indicated by the presence of mitotic figures are not as numerous, apparently, in splenic

lymphatic tissue as in other forms of lymphatic tissue. The regions where mitotic figures are most numerous are, therefore, not points of such special interest and significance as in other cases. Whenever mitotic figures were found, however, they were never located in the central areas, but in the denser peripheral portions of the nodules. There seemed to be no special points, at which the production of lymphocytes by mitosis was very active, and absolutely no evidence of such activity in the lighter central portions, so that the statement of Maximow ('09) and others, "that it is now a commonly accepted fact that small lymphocytes are formed by division of large lymphocytes in the germ centers," certainly cannot be applied to these areas in the lymphatic nodules of the spleen.

In examination of the lighter colored central areas in lymphatic tissue in other locations it has been found that the different concentration or character of the cells is not alone responsible for their appearance. Flemming noted large free cells in these places, similar to large lymphocytes, containing various deeply staining bodies in their cytoplasm, the 'tingible Körper.' Downey and Weidenreich ('12) also found here large cells, derived from reticular cells, which possessed phagocytic powers. Hartmann ('14) saw in the centers of intestinal lymphatic nodules large acidophilic cells with inclusions, which he thought were of a lipid nature. These cells I also observed ('21) in similar locations, and termed them acidophilic macrophages. They were considered as further differentiated, degenerating lymphoid hemoblasts, which had acquired phagocytic powers. The inclusions in the cytoplasm were thought to be remnants of nuclei of degenerating small lymphocytes.

Very few cells of such description were found in the nodules of splenic lymphatic tissue. A few large acid-staining cells which resembled the acidophilic macrophages of intestinal tonsils were found. Their nuclei were very similar to those found in the lymphoid hemoblasts, but did not give a typical staining reaction, staining rather with the acid stain. The basic cytoplasm staining reaction of the lymphoid hemoblasts was also changed to an acid reaction. Some intermediate stages could be found. No evidences

of their origin from reticular cells were found, although it might be considered as possible. In a very few instances deeply staining bodies were found in these cells. In some cases evidences of degeneration of small lymphocytes, such as their pyknotic nuclei and partial fragmentation, were found in these centers, and so, as before concluded in the case of inclusions of the macrophages of the intestinal tonsils, the inclusions of these macrophages were thought to be nuclear remnants of degenerating small lymphocytes.

The same significance is given to the large acid-staining macrophages in the so-called 'germinal centers' of the splenic lymphatic nodules, as to those similarly located in intestinal tonsillar tissue. The change in nuclear and cytoplasmic staining reaction from basic to acid, together with the acquisition of phagocytic powers is considered evidence of the further differentiation and degeneration of the lymphoid hemoblasts of these regions.

From these indications it would seem that the so-called 'germinal center' is not a region of special cell proliferation or transformation, but rather a center of degeneration. The fact that the lighter colored centers are present only in quite large nodules, which is associated with a decrease in the blood supply to these centers, the absence of mitotic figures, or a special mother tissue transforming into lymphocytes, together with the evidences of degeneration of small lymphocytes and of lymphoid hemoblasts, all point to an interpretation of these areas as degenerative rather than proliferative centers.

This interpretation certainly seems to be borne out in a study of pathological conditions. As is well recognized, pathological nodular growths, such as tubercular or sarcomatous nodules, when they become very large, have areas of caseous degeneration in the center. Extreme hypertrophy of lymphatic nodules as occurring in various blood diseases is coincident with degenerative changes in their central areas. In the history of infectious diseases involving the palatine or intestinal tonsils, it has been observed that the central portions of the nodules are the first areas to become affected, indicating that these points are areas of

lowered metabolic rate and resistance. It would seem somewhat illogical to consider these centers as areas of unusual proliferation due to excellent nutritive conditions and high metabolic rate and, at the same time, to admit them as possible foci of infection, associated with points of lowered resistance.

The lighter colored central areas, the so-called 'germinal centers,' when found in nodules of the lymphatic tissue of the spleen, are to be considered, then, as areas, in which the size of the nodules has caused a deficiency in the nutritive supply, as shown by the poor vascularity, which has lowered the metabolic rate to such an extent that evidences of degeneration are found, as shown by the presence of degenerative stages of small lymphocytes and lymphoid hemoblasts, and by the lesser density of lymphocytes there. These centers would consequently be points of lowered resistance and become possible foci of infection in the case of bacterial diseases invading the spleen.

The term 'germinal center,' therefore, seems non-descriptive of existing conditions, as it implies that it is a center of proliferation of lymphocytes, or transformation of other lymphoblastic tissue into lymphocytes, rather than a center of degeneration, due to poor nutritive conditions, as might be concluded from a study of these structures as found in the human spleen.

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## PLATE 1

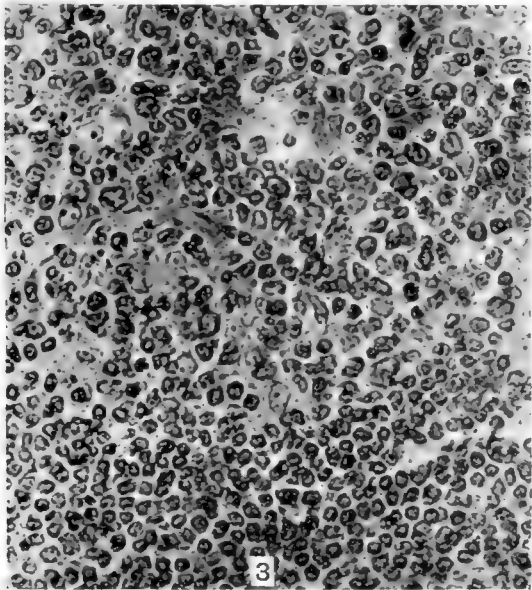
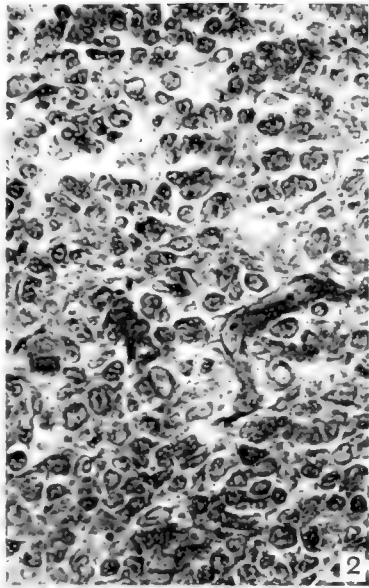
### EXPLANATION OF FIGURES

1 Spleen, Homo, adult, showing a small lymphatic nodule, possessing no lighter colored center, with its accompanying artery located near the center of the nodule.

2 Spleen, Homo, adult, showing the character and density of cells in the lighter colored center of a lymphatic nodule. The reticular tissue is prominent here because of the lesser density of the free cells. Degenerative stages of lymphoid hemoblasts and of small lymphocytes (pycnotic nuclei) may be seen.

3 Spleen, Homo, adult, showing the comparative density of cells in the lighter colored center and in the peripheral portion of a lymphatic nodule. Several cells in various stages of degeneration of lymphoid hemoblasts (lightly staining nuclei) and of small lymphocytes (pycnotic nuclei) are found.





Resumen por el autor, H. S. Colton.

### La anatomía de una rana con cinco patas

El presente trabajo es una descripción de un miembro que surgía en una cintura escapular accesoria situada en el extremo esternal del coracoides y precoracoides derecho. El miembro en cuestión, compuesto de húmero, radio-ulna y una mano reduplicada con ocho dedos, carecía de pigmento y músculos, excepto sobre la cabeza humeral, y aparentemente carecía de sensibilidad. El músculo pectoral del lado derecho presenta su origen en el húmero accesorio y la cintura escapular accesoria, insertándose sobre estas estructuras y no sobre el esternón, como ocurre en el lado izquierdo, que es normal. A causa de la curiosa musculatura, el autor llega a la conclusión de que la anormalidad fué causada por una lesión del esternón.

Translation by José F. Nonidez  
Cornell Medical College, New York.

## THE ANATOMY OF A FIVE LEGGED FROG

HAROLD SELLERS COLTON

*University of Pennsylvania*

FOUR FIGURES

In December 1921 I purchased from a collector of frogs a specimen of *Rana pipiens* with an extra fore limb protruding from the region of the right coracoid. Since it is by studying the abnormal structures which sometimes appear in animals that we are able to shed light on normal conditions, I thought it worth while to study the relation of this limb to the body of the animal.

While polymely is fairly common in the hind limb of the frog<sup>1</sup> it is much rarer in the fore limb. Why this difference exists is perfectly clear. The limb buds of the fore limb, growing as they do in the protected opercular chamber, are not subject to accident as are the buds of the hind limbs, exposed at all times to external conditions. Nevertheless, as can be seen from the table (page 248), polymely of the fore limb is not uncommon.

The frog in question, a small male (five cm. long) was kept alive in an aquarium. During the winter he fed upon spiders, meal worms and beetles. On April 20th he died from some unknown cause, perhaps by the water becoming too hot as the aquarium stood in a sunny place. While he was alive I had abundant opportunity to observe the actions and reactions of the supernumerary limb.

This limb armed with eight digits he kept folded across his chest (fig. 1). Except for the region on the proximal end of the limb no sign of pigment nor any musculature was visible. Indeed, pinching the limb with forceps showed that it was without sensation—no reaction could be observed. Indeed, so numb was

<sup>1</sup> Bateson, W. 1894 Materials for the study of variation. Lond. Macmillan.

TABLE 1  
*Cases of polymely of the forelimbs of Anura*

AUTHOR	DATE	SPECIES	SYMMETRY	REGION	NUMBER OF DIGITS	SHOULDER-GIRDLE SUPERNUMERARY
Superville <sup>1</sup>	1740	?	Right	Scapular	?	?
Gervais <sup>1</sup>	1864	<i>Pelobates cultripes</i>	Left	Scapular, posterior	3	Coracoid and scapula
Lunel <sup>1</sup>	1868	<i>Rana viridis</i>	Left (pair)	Scapular (?)	4 + 4	
Royal College of Surgeons <sup>1</sup>	1872 <sup>2</sup>	?	?			
Mazza <sup>1</sup>	1888	<i>Rana esametica</i>	Left (pair)	Sternal	?	?
Bland Sutton <sup>1</sup>	1889	<i>Rana temporaria</i>	Left	Scapular	3	Scapula and coracoids
Bergendal <sup>1</sup>	1889	<i>Rana temporaria</i>	Left	Scapular	2	None
Tornier	1898	<i>Rana esculenta</i>	Right (pair)	Scapular, one ventral, one anterior	4	Coracoids
Washburn <sup>1</sup>	1899	<i>Bufo columbiensis</i>	Left	Scapular anterior	7	None
Johnson <sup>1</sup>	1901	<i>Rana palmipes</i>	Left and Right	Scapular	4	?
"		<i>Rana hibernum</i>	Right (pair)	Scapular (ventral)	? destroyed	?
Ergerman and Cox	1901	<i>Rana pipiens</i>	Right, ventral	Scapular	?	?
O'Donoghue	1910	<i>Hyla aurea</i>	Left, dorsal	Scapular	5	?
"		<i>Rana temporaria</i>	Right, dorsal	Scapular	Destroyed	Scapula
Wagner	1913	<i>Rana pipiens</i>	2 right 1 left	Sternal	1 4 + 4 + 4	None ?

<sup>1</sup> O'Donoghue, Chas. H. 1910 Instances of polymely in two frogs. Zool. Anz., vol. 35, pp. 759.

the limb that small pond snails attacked it. At times, they covered the limb, apparently feeding upon the epidermis and causing abrasions. The striking characteristics of the supernumerary limb were its position on the ventral side, the lack of musculature, the lack of pigment, and the lack of sensation.



Fig. 1 Photograph of frog, ventral surface. In the dark area anterior to the extra limb there is no muscle layer between the skin and the peritoneum; one looks directly into the body cavity.

The attack of the snails indirectly led to the frog's death. To protect him from the snails I removed him to an aquarium standing before a south window, and without land upon which to crawl. The next morning I found him dead. After his death I preserved him and dissected the arm. The shoulder

girdle proved quite normal on the left side and quite abnormal on the right. The right fore limb was normal, but the coracoid and precoracoid, instead of reaching to the sternum, were very short and supported a mass of cartilage with three centers of ossification. Of the three parts two, in their relationships, suggested coracoid and precoracoid, while the third, a forked structure which slid back and forth on a process from the sternum (fig. 2), could hardly be homologized with the scapula. Attempts to homologize these three parts with the normal shoulder girdle have proved unsatisfactory. With a glenoid cavity in the supernumerary shoulder girdle the extra humerus is articulated. On the distal end of this humerus is an indication of a joint, but no movement is possible. At this point the radio-ulnar is stiffly joined to the humerus. At the distal end of the radio-ulnar a mass of cartilage represents the carpals. From this mass eight digits extend, forming a reduplicated hand. The first digit is present as one, the metacarpal of the second is split on the end, the other three digits are all paired. Figure 3 shows the palmar surface of the double hand. Symmetrical reduplications of a similar sort have been reported by Harrison ('21) in his transplanted limb buds in Salamanders.

While the osteology is interesting the musculature throws more light on the origin of the limb. As I mentioned earlier, the only muscles present in the limb were found at its proximal end, the hand, the forearm and three quarters of the upper arm showed no trace of musculature that I could observe. Four muscles were concerned with the accessory shoulder girdle and the accessory humerus. Three of these muscles (fig 4 *A*, *B*, and *C*) were inserted in the crista deltoidea of the normal right humerus in the position of the normal three portions of the pectoralis muscle, which was absent. The anterior of these three muscles (*A*) had its origin on the accessory shoulder girdle, on the sternum, and on the supernumerary humerus. The second muscle (*B*) had its origin on the accessory shoulder girdle, while the third or posterior muscle (*C*) had its origin on the humerus of the supernumerary limb. A fourth muscle (*D*), in position a continuation of the second (*B*), had its origin on the accessory shoulder girdle

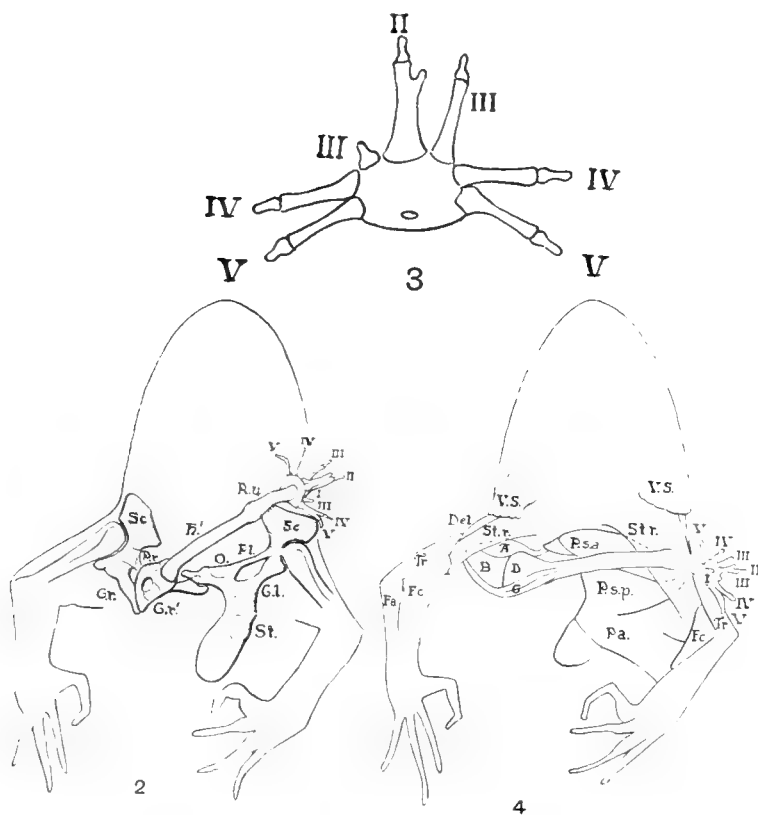


Fig. 2 Osteology of supernumerary limb, twice natural size.

*C.l.* Left coracoid

*C.r.* Right coracoid

*C.r'.* Supernumerary coracoid

*H.* Humerus

*H'.* Supernumerary humerus

*O.* Omosternum

*P.l.* Left precoracoid

*P.r.* Right precoracoid

*R.u.* Radio-ulnar

*Sc.* Scapula

*St.* Sternum

Fig. 3 Palmar surface of hand of supernumerary limb. This shows a twinning on an axis through the first and second digits. The first digit is hidden by the second. Four times natural size.

Fig. 4 The musculature of the fore limbs of the five legged frog. Twice natural size. *A*—Anterior muscle of the accessory limb. *B*—Median muscle of accessory limb. *C*—Posterior muscle of accessory limb. *D*—Muscle connecting supernumerary limb and shoulder girdle. *Del.*—Deltoid. *F.a.*—Flexor antibranchii. *F.c.*—Flexor carpi. *P.a.*—Pectoralis portio abdominalis. *P.s.a.*—Pectoralis portio sternalis anterior. *P.s.p.*—Pectoralis portio sternalis posterior. *St.r.*—Sterno-radialis. *Tr.*—Triceps. *V.S.*—Vocal Sack.

and passing over the head of the supernumerary humerus inserted on that bone. A comparison between the right or abnormal half of the frog with the left or normal half shows that the supernumerary limb is served by muscles homologous to the three portions of the normal pectoralis muscle,—portio sternalis anterior (fig. 4, *P.s.a*), portio sternalis posterior (*P.s.p.*), and portio abdominalis (*P.a.*). Ecker ('89). Supporting this view I found that on the right side the normal pectoralis muscle was lacking, a fact which in life gave the frog a very peculiar appearance. In front of the supernumerary limb a dark patch was visible; there being no layer of muscle between the skin and the peritoneum, one looked directly into the coelom.

A review of cases of fore limb duplication (table 1) shows that the most common type arises in the scapular region and seems to be caused by a split in the limb bud. In two cases, in which, however, the anatomy was not studied, the limb is reported on the ventral side in the region of the sternum. Supernumerary fore limbs then in the Anura may be found in two regions, in the scapular region and in the sternal region.

Speculations on the possible origin of sternal limbs may be based on three sets of evidence; on the osteology, on the musculature and on the nerve supply. The limb may have been caused by a split in the limb bud involving the coracoid and pre-coracoid or it may have been due to an injury to the sternum. The osteology of our limb favors both hypotheses (fig. 2). Since the pectoralis muscle is normally innervated through the brachial nerve, were it possible to trace out the nerve to the pectoralis muscle, it would also favor both hypotheses, therefore a choice between the hypotheses depends upon the musculature. Since the origin of the pectoralis muscle is normally on the sternum, it would seem that the accessory limb has in some way been derived from the sternum. How this may take place is left for experimental analysis.



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Resumen por el autor, S. Naccarati

Sobre la relación entre el peso de las glándulas de secrección interna y los pesos del cuerpo y cerebro.

En el presente trabajo se consigna el peso de las glándulas de secrección interna de cinco especies de reptiles de la fauna italiana, a saber: *Testudo graeca*, *Emys europaea*, *Tropidonotus natrix*, *Zamenis viridi flavus* y *Lacerta viridis*. Los pesos absolutos de las glándulas, incluyendo también los del hígado, páncreas, riñones y bazo, y sus valores relativos con respecto al peso total y al del cerebro, representan la media de un número de animales examinados.

Translation by José F. Nonidez  
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## ON THE RELATION BETWEEN THE WEIGHT OF THE INTERNAL SECRETORY GLANDS AND THE BODY WEIGHT AND BRAIN WEIGHT

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In a previous work (1), in giving the average weight of the thyroid gland of *Emys europaea*, I pointed out that the individual variations are very great. Weibgen (2), Tenchini and Cavatorti (3), Dustin and Zunz (4), and other investigators, who studied the thyroid in man, found also the same great variability.

In this brief report I am giving the absolute weight of the endocrine glands and their value in relation to the body and brain weight in some species of reptiles of the Italian fauna which I had the occasion to study, namely, *Emys europaea*, *Testudo graeca* (Chelonia), *Lacerta viridis* (Lacertilia), *Tropidonotus natrix*, *Zamenis viridi flavus* (Ophidia). These organs show the same variability that was found in thyroid.

Included in this report are also glands of external secretion, which are believed to possess also an internal secretory function.

In my study ovary was omitted because of the great variability due to the physiological condition of the animals. Pituitary, pineal, and parathyroids were not considered on account of the difficulty of estimating them in the small species of reptiles studied by me.

Liver, pancreas, and spleen were in functional activity when the animals were killed.

Weighing operations were conducted within three hours from death.

The relative weight of each organ is referred to the body and to the brain weight in percentage values.

The data I give on reptiles represent averages from a given number of animals. These and especially those given by Welcker and Brandt (5), which are based sometimes on one or two animals, are too few for conclusions of a general nature.

In order to give an idea of the variability I have reported in table 6 the weight of the thyreoid in thirty *Emys europaeas*. Here, and in the case of the other animals, whose tables I have omitted for the sake of brevity, a positive correlation was found between the weight of the animal and that of the thyreoid.

Those who wish detailed and precise data on weights of viscera in man are referred to the works of Reid (6), Peacock (7), Gluge (8), Boyd (9), Bischoff (10), Blosfeld (11), Dieberg (12), Thoma (13), Gocke (14), Oppenheimer (15), Juncker (16), Richet (17), Kress (18), Buchanan and Daly (19), Greenwood (20), Castor (21), Greenwood and Brown (22), Bean (23), Bean and Baker (24).

In the tables, when not otherwise stated, the first column indicates the absolute weights in grams, the second gives the weight of the organ per 100 grams of body weight, the third represents the weight of the organ corresponding to 100 grams of brain substance.

TABLE 1

*Emys europaea* (aver. 30 animals)

1. Body weight	250		
2. Brain weight	0.25	0.1	
3. Thyroid	0.025	0.01	10
4. Thymus	0.08	0.03	32
5. Sex glands (testes, 17 animals)	0.61	0.24	244
6. Adrenals	0.028	0.01	11
7. Spleen	0.16	0.06	64
8. Pancreas	0.28	0.11	112
9. Liver	7.3	2.9	2920
10. Kidneys	1.38	0.5	552

TABLE 2

*Testudo graeca* (aver. 30 animals)

1. Body weight	320		
2. Brain weight	0.3	0.09	
3. Thyroid	0.036	0.011	12
4. Thymus	0.1	0.03	33
5. Sex glands (testes, 20 animals)	0.74	0.23	247
6. Adrenals	0.031	0.01	10
7. Spleen	0.18	0.06	60
8. Pancreas	0.3	0.09	100
9. Liver	8.5	2.65	2833
10. Kidneys	1.55	0.5	517

TABLE 3

*Lacerta viridis* (aver. from 15 animals)

1. Body weight	50		
2. Brain weight	0.121	0.24	
3. Thyroid	0.008	0.016	7
4. Thymus	0.03	0.06	24
5. Sex glands (testes, 8 animals)	0.2	0.4	165
6. Adrenals	0.02	0.04	16.5
7. Spleen	0.08	0.16	66
8. Pancreas	0.2	0.4	165
9. Liver	2.5	5.	2064
10. Kidneys	0.58	1.15	480

TABLE 4

*Tropidonotus natrix* (aver. from 20 animals)

1. Body weight	70		
2. Brain weight	0.1	0.14	
3. Thyroid	0.01	0.014	10
4. Thymus	0.015	0.02	15
5. Sex glands (testes, 12 animals)	0.23	0.3	330
6. Adrenals	0.035	0.05	35
7. Spleen	0.12	0.17	120
8. Pancreas	0.25	0.35	250
9. Liver	2.8	4.	2800
10. Kidneys	0.88	1.4	980

TABLE 5

*Zamenis viridiflavus* (aver. 6 animals)

1. Body weight	220		
2. Brain weight	0.209	0.09	
3. Thyroid	0.045	0.02	21
4. Thymus	0.075	0.03	30
5. Sex glands (testes)	0.48	0.21	230
6. Adrenals	0.08	0.04	38
7. Spleen	0.125	0.06	60
8. Pancreas	0.415	0.18	200
9. Liver	4.825	2.2	2300
10. Kidneys	1.935	0.8	830

TABLE 6

*Weight of thyroid in Emys europaea*

NO.	SEX	WEIGHT OF THE ANIMAL	ABSOLUTE WEIGHT OF THYROID	RELATIVE WEIGHT OF THY- ROID (PER 100 GRAMS OF BODY WEIGHT)
1	F	179	0.025	0.014
2	M	195	0.009	0.005
3	M	200	0.016	0.008
4	M	205	0.020	0.010
5	M	207	0.023	0.011
6	F	220	0.018	0.008
7	F	228	0.029	0.013
8	M	231	0.035	0.015
9	M	232	0.018	0.007
10	F	238	0.026	0.010
11	M	244	0.021	0.009
12	M	246	0.030	0.012
13	M	248	0.025	0.010
14	M	250	0.020	0.008
15	F	255	0.028	0.011
16	F	260	0.025	0.009
17	M	265	0.027	0.010
18	F	271	0.023	0.008
19	F	274	0.033	0.012
20	M	276	0.029	0.010
21	M	279	0.041	0.015
22	F	281	0.032	0.011
23	F	288	0.034	0.012
24	M	290	0.037	0.013
25	F	296	0.038	0.013
26	F	298	0.031	0.010
27	M	309	0.044	0.014
28	M	326	0.039	0.012
29	M	349	0.042	0.012
30	F	375	0.075	0.020

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Resumen por el autor, T. W. Todd.

Significación numérica de las vértebras torácico-lumbares de los mamíferos.

1) Las siguientes ideas aceptadas con relación a la columna vertebral presacra de los mamíferos son indudablemente correctas. El número primitivo de vertebras torácico-lumbares de los mamíferos es y ha sido siempre diecinueve. En la mayor parte de los órdenes de los mamíferos existe una tendencia a aumentar este número (grupo auxispondílico de Welcker). En los Primates la tendencia es hacia la reducción de dicho número, formando los antropoides y el hombre el grupo lipospondílico de Welcker. En los referente a la columna torácico-lumbar, el hombre ocupa un lugar entre los gibones y los antropoideos negros gigantes. 2) Los lemures forman un eslabón entre los Primates y los demás órdenes de mamíferos. Mientras que algunos de ellos exhiben en su especialización un aumento en el número, del mismo modo que los mamíferos auxispondílicos, existen otros que se aproximan a los Primates gigantes típicos en su tendencia hacia la reducción del número de las vértebras torácico-lumbares. La relación de la fórmula vertebral con la especialización de los lemures es muy significativa. 3) Los monos del Nuevo Mundo presentan una tendencia mayor hacia la reducción en número, pero aquí también existe una tendencia marcada hacia el aumento en número, como ocurre en muchos lemures. 4) El número primitivo, diecinueve, es característico de las dos subfamilias de monos antropoides del Antiguo Continente, encontrándose un aumento a veinte lo en raros casos. 5) Los antropoides y el hombre exhiben una reducción progresiva en el número de vértebras, pero en todo este grupo el hombre presenta la condición numérica más estable en su fórmula vertebral. La estabilidad precisa en el hombre está ilustrada de un modo sorprendente en el trabajo reciente de Willis.

Translation by José F. Nonidez  
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# NUMERICAL SIGNIFICANCE IN THE THORACICOLUMBAR VERTEBRAE OF THE MAMMALIA

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## INTRODUCTION

In a study of the vertebral column where the question of anomalies, whether of development or of constitution, must play so prominent a rôle, it is essential that the type, or what is often called the normal, be very clearly understood. And what is equally important, the steps by which this type came into being should be investigated and made plain. Without the latter study one could have no adequate background for a correct understanding of the type. Now the area under discussion, namely, the presacral vertebral column, is defined above by the occiput with the peculiar atlas and axis vertebrae. This special arrangement of the uppermost vertebrae effectively precludes any mistake in identification of the individual bones. Even when the atlas is more or less absorbed into the occipital bone itself, there can be no doubt as to its identity. At the other end the presacral column is defined by the sacrum. Here we do not find an equally clear distinction; there may be an intermediate

form present, defined as a lumbosacral vertebra. For it is well known that the sacrum does not always lie at precisely the same level even in the same species. As the first problem is to inquire into the number of presacral vertebrae and the relation of this number to the phylogenetic position of the various Primates, it is obvious that we must first form a clear impression of the usual constitution of the bones of the presacral column and examine what tendency there may be towards stability of number.

The use of the words *type* and *normal* a little higher up was merely to introduce the reader to the subject in terms with which he is familiar. Both words are, however, frequently used very loosely and in various senses; therefore it is proposed to substitute for them the mathematical expression *mode*, which conveys merely the idea of most frequent occurrence. One would then restate the problem in the following questions: What is the modal number of presacral vertebrae in the various Primates? What stability does this number show? By what steps was it reached?

A review of the mammalian vertebral column, including that of Man, discloses clear evidence of instability of morphological constitution, especially in the cervicothoracic, thoracolumbar and lumbosacral regions. It is the last mentioned which will occupy our attention for the present, although it is impossible to make an adequate study of one area without glancing at times in the direction of the others.

The number of presacral vertebrae in Man is usually asserted to be twenty-four, and the twenty-fifth vertebra is the first sacral according to general acceptance. These statements are only an approximation of the truth: they take no account of the frequent massive variations, and, further, they appear to infer that even in details there is a uniformity which certainly does not exist. We shall make use often of the term presacral vertebrae, and therefore take this occasion to define the expression. Now, of these presacral vertebrae, certain belong to the neck. In mammals these are almost constantly seven in number, the exceptions being beyond our present discussion, although it may be stated in passing that the so-called anomalies in number of

cervical vertebrae do not constitute so marked a stumbling-block to the uniformity of the class Mammalia, or so difficult a problem in phylogeny, as some investigators would have us imagine. We may take it, then, that the number of cervical vertebrae in the mammals generally is seven. Hence, if we know the total of presacrals, we can readily ascertain the number of vertebrae comprising the thoracic and lumbar series together. Since the sole distinction between these series is the possession of the ribs by the former, and since there is marked instability in the exact morphology of the thoracolumbar junction, by which statement we mean that there may be some difficulty in ascertaining exactly at what point one series ends and the other begins, and whereas we desire above all to keep our immediate problem as free as possible from confusing complications, we shall find it convenient to avoid making a distinction between thoracic and lumbar regions and shall therefore consider the vertebrae of both together as the thoracolumbar series. From the earlier part of this paragraph it is plain that the conventional number of thoracolumbar vertebrae in Man is seventeen. Now, since we shall not introduce without specific mention into our discussion the vertebral formula of any mammal which has other than seven cervical vertebrae, we may take it that a variation in the total number of the thoracolumbar vertebrae infers a change in the position of the lower (or hind) limb relative to the vertebral column.

There is another point which must receive preliminary emphasis, namely, the sacrum. The sacrum is merely a composite bone formed by the fusion of certain vertebrae. The factors which, in the aggregate, influence this fusion lie, for the most part, beyond our present consideration. The number of vertebrae comprising the sacrum has no value for this study. The number of vertebrae belonging to and forming the part of the sacrum which articulates with the os innominatum, on the other hand, is of the most absorbing interest, and it is expected that a later communication will convey the results of this study. We consider, at the moment, not the center of articulation between vertebral column and hind limb, but the cephalic delimitation of

this articulation. When we say that the twenty-fifth vertebra is the first sacral we mean that the cephalic delimitation of the articulation with the hind limb is to be found in the twenty-fifth segment from the occiput.

Whereas the number of cervical vertebrae in mammals is commonly known to be practically constantly seven, it is not so apparent that the number of thoracic and lumbar vertebrae is remarkably constant among the Mammalia, diverse though they are in their several bodily conformations, habits, and modes of progression. The following table is taken from Gregory's suggestive memoir upon mammalian orders (7):

	THORACICOLUMBAR VERTEBRAE (AVERAGE)
Monotremata .....	19-20
Marsupialia .....	19
Creodonta .....	20
Carnivora .....	20
Lemuroidea .....	19-20
Rodentia .....	19
Artiodactyla .....	19-20
Insectivora:	
Tupaia .....	19-20
Gymnura .....	20
Microgale .....	22
Solenodon .....	19-20
Sorex .....	20
Galeopithecus .....	18-19

. It must be understood that this table conveys only the most general impression of the number of thoracicolumbar vertebrae in several mammalian orders. The purpose of the moment is simply to draw attention to the comparative frequency of occurrence of the number of 19-20 among mammals, and at the same time to indicate, in the Insectivora, how specialization or divergence from the stem group is associated with a number differing from that which is most frequent in occurrence and most characteristic of the class.

Just as the typical mammalian number of cervical vertebrae is seven, so the number of vertebrae in the thoracicolumbar series of the primitive mammal is either nineteen or twenty; this variation representing the usual limits of divergence. A prim-

itive mammal is one which has retained archaic features in marked degree. It is obvious that any particular mammal may be primitive in certain features, but not in others.

We may assume that nineteen is the primitive number of thoracicolumbar vertebrae in the mammal and that the total primitive number of presacral vertebrae is twenty-six ( $19 + 7$ ). Therefore, the first sacral vertebrae in the primitive mammal is the twenty-seventh.

With these preliminary definitions of the primitive mammalian column clearly in mind, it is proposed to examine the vertebral formulae of the various Primates in order to ascertain, if possible, what has happened to the lower part of the presacral series during the evolution of the creature Man.

#### HISTORICAL ASPECT OF PHYLOGENETIC SHORTENING OF THE PRESACRAL COLUMN IN MAN AND OTHER PRIMATES

That there has been in Man a phylogenetic shortening of the presacral column has been recognized for a very long time. When Sir Richard Owen was Conservator at the College of Surgeons' Museum, comparison of the number of presacral vertebrae in Man and in other mammals formed one of the points of his discourses. At that time one, Holmes Coote, a demonstrator of anatomy at Saint Bartholomew's Hospital, fell under the magnetic influence of Owen, and, with the encouragement of the Master, attempted to introduce into the regular undergraduate courses in anatomy for the several examinations of the College those principles of comparative anatomy which he had absorbed in Owen's lectures. Thus it happened that in 1849 Coote published a work entitled "Homologies of the Human Skeleton," not professing to be original in any way, but merely to bring before the London students, as best he might, the views and researches of his instructor. The book is poorly written and is plainly the work of one who has enthusiastically jotted down during a lecture the fragmentary information which he gleaned in the moments which could be spared actually to listen to the words of the lecturer. The sequence of the paragraphs is disconnected; there is many a hiatus which can indeed be filled in by one who

is acquainted with what Owen was likely to say, but not by the students for whom the volume was written. At the beginning of the second chapter (1, pp. 25, 26) there is an effort to present Owen's views upon the significance of the number of thoracicolumbar vertebrae. This is perfectly clear to those who know their Owen (16, especially vol. 2, pp. 493-494 and 512). Coote meant to convey the idea of significant uniformity in the total number of thoracicolumbar vertebrae among the Carnivora as opposed to the double series (i.e., Artiodactyla and Perissodactyla) among ungulates; that the number, with certain clearly explicable exceptions, is twenty among the Carnivora; and that, taking the number in Carnivora as a basis, there is continuous reduction throughout the Primates until the anthropoids and Man are reached. This idea undoubtedly became very well fixed in the minds of anatomical teachers, and one of the evidences of its propagation is to be found in the attempt by Paterson (17) to prove a tendency towards elongation of the presacral column rather than towards reduction, with references to anthropoid apes and 'quadrupeds.' The conception of an evolutionary shortening of the presacral column in Primates through transmutation and not by suppression is carried on by Keith (13) in an article full of information and suggestion, published in 1902. The same idea formed the basis of the first communication of Rosenberg in 1876 (18). This, indeed, was the earliest concrete formulation of the conception generally held, and as such has rightly come in for a full share of recognition. Subsidiary additions made by Rosenberg at later dates do not carry equally convincing force.

Five years later, Welker, disagreeing in some points with Rosenberg, differentiated between two types of mammals. The primitive presacral number of twenty-six vertebrae is retained in most mammals. Among these there is seen to be a tendency towards lengthening of the column. In marked contrast stands another type of mammal—the tailless apes and Man—in which occurs a shortening process. These Welker termed lipospondylous, in contradistinction to the other group of auxispondylous type (19).



There is, then, nothing new in the perception of a phylogenetic shortening of the presacral column in Primates. As the introduction to a comprehensive study of the vertebral column in man upon a material of hitherto unattainable amount and completeness, of which one contribution is already in the press (20), it becomes necessary to examine this theory in detail and especially in reference to evidence of a phylogenetic nature afforded by other parts of the body.

#### THE THORACICOLUMBAR VERTEBRAE IN LOWER MAMMALS

The Monotremata are the most ancient survivals among mammals, and it is therefore important to notice their presacral formula. Gregory quotes Howes as identifying nineteen thoracicolumbar vertebrae in both genera, namely *Ornithorhynchus* and *Echidna* (except *Proechidna* which would have twenty) (7, p. 152), and continues: "But in the view of the writer the anterior sacral is only a modified lumbar and the posterior sacral only a slightly modified caudal. In that case the vertebral formula would be as follows:

"Ornithorhynchus C.7, T.17, L. 2, S.1, CS.1, Cd.19;

Echidna C.7, T.16, L. 5, S.2, —, Cd.10."

In tracing down this reference I find that the citation of Howes' name is certainly a slip of the pen. The reference is really to Flower's *Osteology* (6). Now it appeared to me that the statement of Gregory might indicate some instability occurring in the lumbosacral region of these lowly and very specialized mammals, primitive though they are in many particulars. I have therefore studied closely the three skeletons in the Hamann Museum. Our *Ornithorhynchus*, B.174, shows no sign whatsoever of ambiguity in the last lumbar or first sacral vertebra. It is true that the last (third) sacral vertebra might very well be called caudal, although there are sufficient points of distinction to make clear its appropriate position. Only two sacral vertebrae take part in the articulation with the ilium. Therefore, the vertebral formula as I would state it for this example is:

C.7, T+L.19, S.2+1, Cd. —.

Our two examples of *Echidna* are both *E. aculeata*. Neither shows the least sign of ambiguity in the lumbosacral region. In both the upper three pieces of the sacrum articulate with the ilium and for both the vertebral formula is:

C.7, T+L.19, S.3+1, Cd. —.

I would make the same reservation for the last sacral in these skeletons as in the case of our *Ornithorhynchus*.

In order to enlarge the material upon which conclusions of sufficient accuracy may be drawn, I have referred to the Catalogue of the College of Surgeons (5). In this catalogue Flower cites five examples of *Ornithorhynchus* skeletons, all of which show a thoracolumbar column numbering nineteen vertebrae. In each case also the sacrum was formed of three vertebrae, though naturally it is not stated how many of these articulated with the ilium. There are four skeletons of *Echidna*, all of which possess nineteen thoracolumbar vertebrae. Two have three sacral vertebrae and two have four.

Now, in the face of these facts, one can only infer that the skeletons examined by Gregory were unusual in the conformation of the lower lumbar column and that it is certainly more correct to describe the *Monotremata* as possessing nineteen thoracolumbar vertebrae.

We note that this number nineteen is practically constant also for the *Marsupialia*, the next most ancient group of mammals.

Turning to the placental mammals, we would naturally first inquire the condition in the insectivores because of the central position of this order, and in the *Rodentia* which also represent a very ancient and, in many ways, relatively little changed order.

The importance of these orders in a study of other mammals was emphasized many years ago by Huxley. "Given the common plan of the *Insectivora* and of the *Rodentia*, and granting that the modifications of the structure of the limbs, of the brain, and of the alimentary and reproductive viscera which occur among them may exist and accumulate elsewhere, and the derivation of all the *Eutheria* from animals which, except for their simpler placentation, would be *Insectivores*, is a simple deduction from the law of evolution" (12, p. 657).

For a general survey of the vertebral formula there is no better reference than the Catalogue of the College of Surgeons (5), for both Owen and Flower paid attention to this matter. One has only to turn the pages of this catalogue to be impressed with the remarkable constancy of the number nineteen in the thoracico-lumbar column of rodents. A certain number of very specialized genera, it is true, show a somewhat greater number, and here and there an odd individual may possess twenty in the vast majority of the genera in which nineteen thoracicolumbars are typical. This is quite what one would expect and merely serves to confirm the assertion that nineteen is the general mammalian number.

The insectivores present a rather different case. All of these are specialized, some very greatly. Yet even among insectivores the less specialized and aberrant possess the typical thoracico-lumbar number. The lipotyphlous insectivores should of course be distinguished from the Menotyphla.

The total thoracicolumbar number in the insectivores is unusually unstable. For example, the number in *Gymnura*, the genus specially picked out by Huxley for its importance as a relatively undifferentiated Eutherian, has twenty according to Flower (6, p. 86), twenty according to the Catalogue (5, p. 647), but twenty-one (i.e.,  $16 + 5$ ) in our specimen B 545.

Of all existing insectivores the Tupaiidae, or Tree Shrews of the Orient, probably show most faithfully the conditions reminiscent of the stem placentals. For a full discussion of the probable ancestry of the mammals with significant details of the habits and mode of life of these, as yet largely hypothetical, creatures the reader is referred to the article by Matthew on the arboreal ancestry of the Mammalia (14). The well-established belief that *Tupaia*, besides being one of the most generalized insectivores, lies not far from the point of divergence of Primates and insectivores, renders this animal of special interest, and Gregory's remarks (7, p. 288) are particularly suggestive.

Of existing Menotyphla the skeleton of the arboreal Tupaiidae appears far more generalized, especially in the limbs and backbone, than does that of the terrestrial, saltatorial Macroscelididae.

There is also considerable indirect evidence for ascribing to the stem Insectivores semi-arboreal insectivorous-omnivorous habits.

Such habits seem the best fitted to give rise by adaptive radiation to all others. The habit of running along the branches, of jumping from branch to branch, favors an even development of all the muscles of the limbs, hands and feet and puts a premium on a high average development of mental faculties; whereas aquatic, fossorial, cursorial, saltatorial and volant habits all imply limitation of movement of the limbs in particular directions, and the hypertrophy of certain parts at the expense of others, with resultant one-sided specialization of the nervous system. This is fully illustrated in the various types of terrestrial, aquatic and fossorial Insectivores, which are all highly specialized in these directions; whereas the trunk and limbs of the arboreal Tupaiidae are distinguished not only by the almost entire lack of hypertrophy of one part over another but also by the retention of very numerous characters (such as a free centrale carpi, a third trochanter, entepicondylar foramen, pentadactyly, etc., etc.), which, on any theory, are admitted to be primitive mammalian characters.

The semi-arboreal habit also favors the retention of small size and it is obvious that the opposite condition, increasing size and weight, means larger muscles and greater need for the development of special processes on the bones; this tends as it were, to upset the balance of form-determining forces and to start new or peculiar lines of specialization.

Our single skeleton of *Tupaia tana*, B. 211, has the following vertebral formula:

C.7, T+L.19, S.1+2, Cd. —.

The thoracicolumbar column retains the primitive number of pieces, and an equally significant fact is that the first piece of the sacrum alone articulates with the ilium, although the sacrum consists of three pieces. Thus *Tupaia* shows an articulation with the ilium more primitive in character than even the monotremes. Nineteen thoracicolumbars is the number accorded by Gregory from a specimen in the National Museum, and it is the number in the College of Surgeons specimen (5, p. 649). But Gregory cites evidence of a variation in number from eighteen to twenty (7, p. 275).

The other Tree Shrew, by name *Ptilocercus*, not so lemur-like as *Tupaia*, also presents nineteen thoracicolumbar vertebrae (9). On the other hand, the terrestrial Macroscelididae show the beginning of the usual mammalian tendency to increase this number. Gregory quotes de Blainville and Mivart to the effect that *Macroscelides* has either nineteen or twenty thoracicolum-

bars (7, p. 282), and Peters as giving twenty-one to *Rhynchoeyon*. The College of Surgeons specimen of *Rhynchoeyon* also presents twenty-one (5, p. 649).

#### THE PRIMATE VERTEBRAL COLUMN

We pass now to consider the Primate vertebral column, bearing in mind that the stem Primates diverged from the common insectivorous-omnivorous stem placentals when the latter were in a stage of bodily formation probably not so very unlike our modern Tree Shrews. Thus in all likelihood the number of the thoracicolumbar vertebrae in the ancestral stem Primates was nineteen.

#### THE THORACICOLUMBAR VERTEBRAE OF THE LEMURS

Upon the assumption that the further back in the history of any form of animal one goes the more likely one is to discover the primitive or parent type, we glance at the paleontological story of the Primates. There is no doubt that the lemurs represent the earlier and more primitive form and that in many respects the New-World monkeys retain lemur-like features.

The Old-World Primates higher than the lemurs are structurally much further removed from the lemurs. The Old-World apes differ in many respects from the anthropoids and Man, so that it is better to keep them separate in mind, merely with the small anthropoid *Hylobates*, the Gibbon, as a connecting link.

Now the lemuroid characters of *Tupaia* may be due to convergent evolution, but, as Gregory says, "the provisional conclusion is that the Tupaiaidae are descended from the Insectivore stock that gave rise to the Primates" (8). We are to examine the Primate history therefore with the features of *Tupaia* in mind, recalling especially the primitive number of thoracicolumbar vertebrae.

The most ancient lemur known is the Eocene form *Notharctus*, the skeleton of which is considerably more primitive than that of any later Primate (Gregory, 10). Unfortunately, the complete vertebral column is not known, but the skeleton so closely resembles that of modern lemurs in principle, though not in proportions, that we may feel confident in attributing to *Notharctus*

the same number of thoracolumbar vertebrae as in existing species. After all, this is really the important feature of the search for an ancestral form; the modern species have probably not traveled far in their skeletal pattern from their progenitor. As in Lemur, the articular surface for the ilium is borne almost entirely by the first sacral vertebra (Gregory, 11). This is also evidence of the probable identity of the vertebral formula of Notharctus with that of Lemur. Some lemurs retain a greater number of primitive characters than others; some lemurs are very specialized and aberrant. Therefore, it will not do for us to take an average over the thoracolumbar columns of many individual specimens; our specimens must be arranged. For this reason, among others, the well-known work of Le Double has been of singularly little service to me. Indeed, I am strongly of the opinion that it is actually misleading unless used with precaution. I have extracted the records of vertebral formulae from the Catalogue of the Royal College of Surgeons (5) and added these to our own records here with the following results.

There is nothing more distressing and time-consuming than the attempt to identify exact species and sometimes even genera of Primates from the data given by most authors. This confusion has grown up more in reference to the brain than in other morphological fields. But even in the skeleton it is not wanting. In order to avoid possible misunderstanding, I have named all the specimens in the following pages in accordance with D. G. Elliot's monograph, which in spite of certain shortcomings is the most generally useful standard for reference (2).

*Lemurinae*

Lemur:

L. catta Linnaeus.	T+L 20	W.R.U.,	B. 137.
L. catta Linnaeus.	T+L 19	W.R.U.,	B. 197.
L. catta Linnaeus.	T+L 18	R.C.S.,	265.
L. variegatus Kerr.	T+L 19	W.R.U.,	B. 344.
L. variegatus Kerr.	T+L 19	R.C.S.,	262.
L. albifrons? E Geoffroy.	T+L 19	R.C.S.,	270.
L. mongoz? Linnaeus.	T+L 19	R.C.S.,	271.
L. mongoz? Linnaeus.	T+L 19	R.C.S.,	272.
L. sp.	T+L 19	W.R.U.,	B. 138.

Lepidolemur:

L. ruficaudatus Grandidier.	T+L 21	W.R.U.,	B. 345.
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*Chirogaleinae*

Microcebus:			
M. murinus Miller.	T+L 20	W.R.U., B.	434.
M. murinus Miller.	T+L 20	R.C.S.,	281.
M. furcifer Blainville.	T+L 20	R.C.S.,	279.
Myoxicebus:			
M. griseus E. Geoffroy.	T+L 18	R.C.S.,	260.

*Indrisinae*

Propithecus:			
P. diadema Bennett.	T+L 20	R.C.S.,	255.
Indris:			
I. indris Gmelin.	T+L 21	W.R.U., B.	343.
I. indris Gmelin.	T+L 21	R.C.S.,	252.
Lichanotus:			
L. laniger Gmelin.	T+L 21	R.C.S.,	258.

*Daubentoniidae*

Daubentonia:			
D. madagascariensis Gmelin.	T+L 19	W.R.U., B.	198.

*Lorisiae*

Perodicticus:			
P. potto E. Geoffroy.	T+L 21	R.C.S.,	294.
Arctocebus:			
A. calabarensis Smith.	T+L 22	R.C.S.,	297.
Loris:			
L. tardigradus Linnaeus.	T+L 23	R.C.S.,	288.
L. tardigradus Linnaeus.	T+L 23	R.C.S.,	289.
L. tardigradus Linnaeus.	T+L 23	R.C.S.,	291.
Nycticebus:			
N. borneanus Lyon.	T+L 23	W.R.U., B.	136.
N. javanicus E. Geoffroy.	T+L 23	R.C.S.,	292.

*Galaginae*

Galago:			
G. crassicaudatus E. Geoffroy.	T+L 19	R.C.S.,	283.
G. alleni Waterhouse.	T+L 19	R.C.S.,	287.

*Tarsiidae*

Tarsius:			
T. borneanus Elliot.	T+L 19	W.R.U., B.	135.

Although the combined series of lemurs here presented is not large, it is comprehensive enough to be exceedingly suggestive. The frequent recurrence of nineteen thoracicolumbar vertebrae cannot be without significance, especially when one recalls that this number is characteristic of all primitive mammals. Our

knowledge of the history and the relationships of the lemurs makes us accept unhesitatingly the inference that the number nineteen is equally indicative of a primitive character in these animals. But there are numerous deviations from the number nineteen, and these with two exceptions are in the direction of a higher number. We have seen the same tendency in insectivores and it is duplicated in other mammalian orders. The Perissodactyla, for example, have all deviated in the direction of a longer thoracolumbar column. That the number increases, and that the increase should be within strict limits, indicates the phylogenetic or evolutionary trend. That a similar increase should be found among many varied types of mammal is what we should expect from the general principles of parallel evolution. Now, if we simply say that among twenty-nine lemurs the number of thoracolumbar vertebrae is nineteen in 11 cases, eighteen in 2, twenty in 5, twenty-one in 5, twenty-two in 1, and twenty-three in 5, we shall fail altogether to grasp the real significance of the variation. In order to appreciate properly the trend of the variation, we must examine the relationships of these genera and species to each other.

To define adequately the degree of affinity of one animal to another is no easy matter, for it is naturally purely relative, and about the precise position there may be some reasonable difference of opinion. Nevertheless, the marshalling of these lemurs may be attempted in a general way. We shall take as our guide, in the first place, the characters of the lemur brain as set forth by Elliot Smith (4), and, secondly, certain unpublished notes made by myself upon the skull and teeth of the lemurs for a forthcoming work.

Now, of all the lemurs *Tarsius* is undoubtedly the most primitive, although not far from it in primitive characters come the galagos. Closely related to the galagos, but more specialized are the pottos (*Perodicticus*), and still more specialized are the genera *Loris* and *Nycticebus*, which are intimately related one to another, frequently confused in description, and imperfectly separated zoologically. If, now, the table of vertebrae be consulted with the foregoing general statement in mind, the wonder-



fully accurate manner in which the increase in length of the thoracolumbar column keeps pace with the general evolutionary trend appears most striking. The number nineteen in *Tarsius* and the galagos gives place to twenty-one and two in the pottos and to twenty-three in *Loris* and *Nycticebus*. I am aware that I am not giving in detail the arguments of Elliot Smith on the brain or my own on skull and dentition. The former can be obtained from the original paper (4) and the latter will be available later. But it is rather striking that work carried out upon parts of the body so distinct as brain on the one hand and teeth and skull on the other gives such harmonious results.

As to the genus *Lemur* itself, there is very little comment to make. All the species are much alike in their anatomical features. They are less primitive than *Tarsius* or *Galago* but, in spite of their greater size, retain many of the primitive features of brain and skull. Elliot Smith says: "The cerebral features of the members of the genera *Lemur* and *Hapalemur* (i.e., *Myoxicebus*) are not far removed from those of *Galago*." Nineteen is their characteristic number of thoracolumbar vertebrae. *Lepidolemur* also with nineteen thoracolumbar vertebrae is in some ways more specialized towards the pithecoïd type, in other ways more primitive than *Lemur* itself. The single instance in which the thoracolumbar vertebrae of *Lemur* number twenty finds its explanation in the lemuroid tendency to lengthening of the column. The solitary case in which the number is only eighteen can be understood upon reference to the section of this chapter dealing with the non-lemurine Primates.

We now pass to the next group, namely, the *Chirogaleinae*. Of these *Myoxicebus* itself differs very little in its brain from *Lemur*, although it may be that *Myoxicebus* shows a slightly nearer approach to the pithecoïd type. The records of skull and dentition accord almost in detail with this, for my notes state that it shows "altogether about the same amount of specialization as *Lemur* though along a rather different line." As a result, we are quite prepared to find that the thoracolumbar number is eighteen, and not nineteen or twenty. *Microcebus*, unfortunately, receives no clear statement. Elliot Smith points out

similarities to Lemur and to the Lorisinae, but does not commit himself. My notes state in part: "Palate approaching Lemur type. Upper central incisor still large as in Insectivora. Premolars and molars fairly generalized. I am very uncertain about this beast." It may be that *Microcebus* is more specialized than *Myoxicebus* and perhaps somewhat retrogressive. This would accord quite well with the twenty thoracolumbar vertebrae.

Finally we deal with the very specialized families Indrisinae and Daubentoniidae. Towards the former, according to Elliot Smith, *Lepidolemur* points the way. Of these undoubtedly *Propithecus* is least specialized, then *Indris* and, most specialized of all, *Lichanotus*. Again this agrees with the increasing number of vertebrae, namely, twenty in *Propithecus* and twenty-one in the others.

*Daubentonia*, in spite of its retrogressive features, shows clear evidence of its origin from a very Lemur-like type specialized along the lines of the Indrisinae. My notes state that there are some evidences of a pithecoïd tendency. This gives added weight to the rather striking fact that the number of thoracolumbar vertebrae has not increased as in the Indrisinae, but remains nineteen.

I have dealt somewhat at length with the problem of numerical variation of vertebrae in the lemurs because I desire to make clear the argument for a significant and not accidental phylogenetic trend in the vertebral column and thus pave the way for a more definite understanding of the types of variation in the lower lumbar column of man. Matthew, in discussing the species of *Notharetidae* from the successive geological horizons of the lower and middle Eocene, makes the following very illuminating statement (15:

As with other abundant groups, a large series shows a certain range of individual variation, some being more and others less progressive, but within comparatively narrow limits. As we progress upward through successive levels of the formations, we find that the limits of individual variation, on one side or the other of the abundant typical forms, are progressively shifted over in the direction of the phyletic trend. That this is a gradual shifting of averages, due to the disap-

pearance of less progressive individuals, and appearance and increase of more progressive individuals, seems to be fairly well shown in this dhyllum [i.e. *Notharctus* and *Pelycodus*. T.W.T.] . . . . It is not the gradual replacement of one species by another distinct and more progressive species, but so far as one may judge from the characters of the teeth the gradual conversion of one species into its successor by the progressive elimination of the more primitive and increase in numbers of the more advanced individuals.

What we are now in effect looking at are the terminal stages of the progression, one animal after another representing a phase or offshoot along the general phylogenetic trend. In the lemurs we note in the main a trend towards lengthening of the thoracicolumbar column as in most other mammalian orders. But here and there we find a suggestion of another type of trend, namely, towards actual shortening of the column. It is this type, barely suggested in the lemurs, which we shall find ever more and more strongly evidenced as we pass in review the successively higher groups of non-lemurian Primates.

# THE THORACICOLUMBAR VERTEBRAE IN THE NEW-WORLD MONKEYS

If we arrange the columns of the New-World monkeys in order, commencing with those in which nineteen thoracicolumbar vertebrae are characteristic, we find the following notable results:

## Hapalidae:

<i>Oedipomidas oedipus</i> Linnaeus.	T+L 19	R.C.S.,	244.
<i>Callithrix jacchus</i> Linnaeus.	T+L 19	R.C.S.,	246.
<i>Callithrix jacchus</i> Linnaeus.	19	R.C.S.,	247.
<i>Callithrix jacchus</i> Linnaeus.	19	R.C.S.,	249.

## Cebidae:

### Cebus:

<i>C. capucinus</i> Linnaeus.	T+L 19	R.C.S.,	208.
<i>C. capucinus</i> Linnaeus.	20	R.C.S.,	209.
<i>C. capucinus</i> Linnaeus.	19	R.C.S.,	214.
<i>C. capucinus</i> Linnaeus.	19	R.C.S.,	215.
<i>C. unicolor</i> Spix.	T+L 19	W.R.U., B.	142.

## Pithecia:

<i>P. monacha</i> E. Geoffroy.	T+L 19	R.C.S.,	230.
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## Alouatta:

<i>A. palliata</i> Gray.	T+L 20	W.R.U., B.	340.
<i>A. seniculus</i> Linnaeus.	T+L 18	R.C.S.,	235.

## Ateles:

<i>A. geoffroyi</i> Kuhl.	T+L 18	R.C.S.,	217.
<i>A. belzebuth</i> E. Geoffroy.	T+L 17	W.R.U., B.	141.

## Lagothrix:

L. lagotricha Humboldt.	T+L 18	R.C.S.,	226.
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## Saimiri (Chrysothrix):

S. sciureus Linnaeus.	T+L 20	R.C.S.,	202.
S. sciureus Linnaeus.	20	R.C.S.,	203.
S. sciureus Linnaeus.	20	R.C.S.,	204.
S. oerstedii Reinhardt.	20	W.R.U.,	B. 341.

## Aotus (Nyetipithecus):

A. vociferans Spix.	T+L 22	R.C.S.,	227.
A. gularis Dollman.	T+L 21	W.R.U.,	B. 342.

We cannot attempt to follow out in detail the relationships of these animals the one to the other. Suffice is to say that all tend toward specialization of a rather pronounced nature, but that *Cebus* itself occupies a central position among Primates generally (Elliot Smith, 3, p. 399). From the typically primitive mammalian number as shown characteristically by *Cebus*, we note two trends of variation. The one evidenced by *Saimiri* and *Aotus* is towards lengthening, as occurs almost exclusively among the lemurs. The other exhibits a more or less marked tendency towards shortening. This trend was seen only in *Lemur* and *Myoxicebus* among the lemurs. For our present purpose the New-World monkeys are significant in that while their brain shows a very close affinity with that of the lemurs, their vertebral column presents a stage distinctly intermediate between those of the lemurs and of the higher apes.

#### THE THORACICOLUMBAR VERTEBRAE OF THE OLD-WORLD APES

In contradistinction to the two types of trend shown by the *Cebidae*, the thoracicolumbar column of the *Cercopithecidae* presents a remarkable stability in both the subfamilies.

##### *Subfamily Lasiopyginae*

<i>Lasiopyga mona</i> Schreber.	T+L 19	W.R.U.,	B. 147.
<i>Lasiopyga griseoviridis</i> Demarest.	T+L 19	R.C.S.,	112.
<i>Lasiopyga albigularis</i> Sykes.	T+L 19	R.C.S.,	118.
<i>Lasiopyga petaurista</i> Schreber.	T+L 19	R.C.S.,	124.
<i>Lasiopyga</i> sp.	T+L 19	R.C.S.,	108.
<i>Erythrocebus patas</i> Schreber.	T+L 19	R.C.S.,	109.
<i>Pithecius rhesus</i> Audebert.	T+L 19	W.R.U.,	B. 460.
<i>Pithecius rhesus</i> Audebert.	T+L 19	W.R.U.,	B. 148.
<i>Pithecius rhesus</i> Audebert.	T+L 19	R.C.S.,	151.

<i>Pithecus rhesus</i> Audebert.	T+L 19	R.C.S.,	152.
<i>Pithecus rhesus</i> Audebert.	T+L 19	R.C.S.,	153.
<i>Pithecus rhesus</i> Audebert.	T+L 19	R.C.S.,	154.
<i>Pithecus rhesus</i> Audebert.	T+L 19	R.C.S.,	155.
<i>Pithecus nemestrinus</i> Linnaeus.	T+L 19	W.R.U., B.	337.
<i>Pithecus nemestrinus</i> Linnaeus.	T+L 19	R.C.S.,	160.
<i>Pithecus fascicularis</i> Raffles.	T+L 19	R.C.S.,	135.
<i>Pithecus fascicularis</i> Raffles.	T+L 19	R.C.S.,	136.
<i>Pithecus fascicularis</i> Raffles.	T+L 18	R.C.S.,	134.
<i>Pithecus pileatus</i> . Kerr.	T+L 19	R.C.S.,	144.
<i>Pithecus sinicus</i> Linnaeus.	T+L 19	R.C.S.,	145.
<i>Simia sylvanus</i> Linnaeus.	T+L 19	R.C.S.,	170.
<i>Theropithecus gelada</i> Rüppell.	T+L 19	R.C.S.,	178.
<i>Theropithecus gelada</i> Rüppell.	T+L 19	R.C.S.,	179.
<i>Papio cynocephalus</i> Linnaeus.	T+L 19	R.C.S.,	184.
<i>Papio</i> sp.	T+L 19	R.C.S.,	187.
<i>Papio porcarius</i> Brunnich.	T+L 19	R.C.S.,	192.
<i>Papio leucophaeus</i> F. Cuvier.	T+L 19	R.C.S.,	196.
<i>Papio sphinx</i> . Linnaeus.	T+L 18	R.C.S.,	198.
<i>Papio</i> sp.	T+L 19	W.R.U., B.	151.

*Subfamily Colobinae:*

<i>Pygathrix entellus</i> Dufrèsne.	T+L 19	W.R.U., B.	158.
<i>Pygathrix entellus</i> Dufrèsne.	T+L 19	R.C.S.,	83.
<i>Pygathrix entellus</i> Dufrèsne.	T+L 19	R.C.S.,	90.
<i>Pygathrix rubicunda</i> Müller.	T+L 19	W.R.U., B.	622.
<i>Pygathrix schistaceus</i> Hodgson.	T+L 19	R.C.S.,	96.
<i>Pygathrix cristata</i> Raffles.	T+L 19	R.C.S.,	100.
<i>Nasalis larvatus</i> Wurm.	T+L 18	R.C.S.,	105.
<i>Nasalis larvatus</i> Wurm.	T+L 19	R.C.S.,	107.
<i>Colobus vellerosus</i> I. Geoffroy.	T+L 19	R.C.S.,	78.

Now the striking fact to be observed in these animals beyond the constancy of the number nineteen is the fact that when variation does occur it is, in both subfamilies, a reduction to eighteen. We note, then, that no longer is the general mammalian trend towards elongation to be found. In this respect the lemurs are distinctly the link with mammals generally. The *Cebidae* occupy an intermediate position between the far more typically mammalian lemurs and the distinctively primate Old-World apes. We shall see the same sort of intermediate part taken by the small anthropoid *Hylobates* between the giant anthropoids and the Old-World apes.

## THE THORACICOLUMBAR VERTEBRAE IN THE ANTHROPOIDS

In considering the anthropoids one must separate the gibbons from the giant apes. The former are exceedingly like the Cercopithecidae in many respects and, a priori, would be expected to show a vertebral formula close to that of the Old-World apes. The giant anthropoids, on the contrary, are very specialized, more specialized in many ways than in Man himself. The ensuing tables show plainly that the vertebral formula of the several anthropoids confirms these general impressions.

## Hylobatidae:

Hylobates concolor Harlan.	T+L 18	W.R.U.,	B. 160.
Hylobates concolor Harlan.	18	W.R.U.,	B. 162.
Hylobates concolor Harlan.	17	W.R.U.,	B. 161.
Hylobates lar Linnaeus.	18	R.C.S.,	65.
Hylobates lar Linnaeus.	18	R.C.S.,	66.
Hylobates sp.	18	R.C.S.,	63.
Hylobates hoolock Harlan.	17	W.R.U.,	B. 159.
Symphalangus syndactylus Desmarest.	T+L 17	R.C.S.,	58.
Symphalangus syndactylus Desmarest.	17	R.C.S.,	61.

## Giant Anthropoids:

Pan	T+L 17	W.R.U.,	B. 168.
	17	W.R.U.,	B. 346.
	17	W.R.U.,	B. 347.
	17	W.R.U.,	B. 543.
	17	W.R.U.,	B. 632.
	16	W.R.U.,	B. 171.
	16	W.R.U.,	B. 540.
	17	R.C.S.,	1
	17	R.C.S.,	13
	16	R.C.S.,	4
	16	R.C.S.,	5

## Gorilla

T+L 17	W.R.U.,	B. 168.
17	W.R.U.,	B. 239.
17	W.R.U.,	B. 624.
16	W.R.U.,	B. 169.
16	W.R.U.,	B. 626.
16	W.R.U.,	B. 627.
17	R.C.S.,	20
17	R.C.S.,	23
17	R.C.S.,	29
17	R.C.S.,	30
16	R.C.S.,	21

Pongo	T+L	16	W.R.U.,	B.	164.
		16	W.R.U.,	B.	172.
		16	W.R.U.,	B.	623.
		16	W.R.U.,	B.	624.
		17	R.C.S.,		37
		16	R.C.S.,		38
		16	R.C.S.,		40
		16	R.C.S.,		50
		16	R.C.S.,		51

The same process of reduction which is characteristic of the Old-World apes becomes still more striking among the anthropoids. Of the gibbons the less specialized show eighteen or seventeen thoracicolumbar vertebrae and of the two skeletons of the more highly specialized *Symphalangus* each possesses only seventeen. In the giant anthropoids the number seventeen is more characteristic of the great black anthropoids, as it is of Man. In these animals, much more than in Man, there is a tendency to reduction to sixteen. This reduction to sixteen becomes characteristic of the very specialized Orang, Pongo. In this general manner we may read the tables at a glance; it will, however, be profitable to consider them a little more closely.

Keith's table III (13, p. 24) is very illuminating and in point of number greatly exceeds ours. The specimens from the College of Surgeons are included both in my tables and in Keith's. If, then, we reduce to actual numbers the percentages in Keith's table and add those skeletons belonging to the Hamann Museum, a composite statement can be set forward. For the statistics upon Man in Keith's table I have substituted the figures obtained by Willis (20) in this laboratory as being probably less selected. Of course it must be understood that Willis' figures refer to a mixture of White and Negro races and both sexes. For further details the reader should consult the original paper.

There is no doubt about the progressive reduction in number with such a table to hand and there is likewise no doubt concerning the relative position of Man in the progression. Reduction has gone distinctly further in the giant anthropoids and has left Man sensibly nearer the gibbons.

*Number of thoracolumbar vertebrae*

	TOTAL	15	16	17	18	19
Orangs	50	3	39	8		
Gorillas	33	2	13	18		
Chimpanzees	45	1	10	26	8	
Man	748		5	717	26	
Gibbons	63			11	48	4

That the primitive number nineteen is not exceeded in either subfamily of Old-World apes appears both from the Reserve material and from the skeletons in the College of Surgeons. But Keith has indicated that an increase to twenty does occasionally appear in *Pithecus* and more frequently in *Papio*. One would not deny the existence of this increase, relatively infrequent though it is. However, it must be borne in mind that prepared skeletons supplied by dealers or macerated by inexperienced preparators not rarely show evidence of tampering with the vertebral column, which is either composite or lacks one or more vertebrae. I am therefore not prepared to pay much attention to outlying stragglers. Another difficulty met when one attempts arbitrarily to assign vertebral columns to definite figures, which necessarily imply a greater degree of definition than actually occurs in the column itself, is that of the lumbosacral vertebra in its several degrees. There may quite justly be a difference of opinion as to its exact position in the column.

In his investigation of our material Willis found it worth while to separate on the one hand those columns showing distinctly and unquestionably sixteen, seventeen, or eighteen thoracolumbar vertebrae, and on the other the intermediate types. Consequently, he found that the statements as given in the table above by no means represent the real condition of the human column. In addition to the five specimens possessing only sixteen thoracolumbars there were thirty-five showing an abmodal condition approximating sixteen. Also beyond the twenty-six with eighteen thoracolumbars there were fifteen abmodal examples which might well be considered as tending distinctly towards eighteen. If, then, these imperfect types be added to the perfect examples, the distribution is changed to the following:



Sixteen T-L, 40. Seventeen T-L, 667. Eighteen T-L, 41.

Going into this matter still further, Willis finds that, considering each tendency to reach complete definition at 100 per cent, the tendency towards the presumably ancestral eighteen is expressed by 63.4 per cent of the abmodals reaching complete definition and only 36.6 per cent remaining partial or incomplete. The tendency towards sixteen is expressed by only 12.5 per cent reaching complete definition; 87.5 per cent. the great majority, remaining incomplete. Now this is a much happier method of presenting the progression as shown in Man. No doubt if such a scheme could be adopted for the other Primates we should have just as enlightening a description of what has taken place in them. Meantime we must content ourselves with such an imperfect arrangement as I have given in my table above. All that can actually be deduced is that seventeen is the modal number for Man and the giant black anthropoids, whereas eighteen is modal for the small and Old-World ape-like gibbons, and sixteen for the Orang. It may, however, be inferred with some reason that in the Chimpanzee, as in Man, there is about equal tendency towards eighteen and sixteen thoracicolumbars, although from the frequency of expression of this tendency in the Chimpanzee it is apparent that the column of that ape is in a much more unstable condition than the column of Man. The Gorilla has progressed further along the line of specialization and appears to exhibit no tendency towards eighteen. The Orang is fairly definitely settled with sixteen.

The really noteworthy fact is that the column of Man is remarkably stable numerically as compared with the columns of his near relatives, so far as we are able to obtain detailed information. This appears very clearly indeed in Willis' careful discussion.

The relative stability in White and Negro races is a problem to be taken up later. Judging from other studies upon the human skeleton, we must be prepared to find no evidence of a 'lower' or a 'higher' race, but rather a humanoid specialization more marked in the Negro than in the White.

Now, in summary of the foregoing observations, we may note several points. It is not necessary to obtain the entire number of vertebral segments including the tail or coccyx to enable one to come to a decision concerning transmutation of vertebral segments during phylogeny. We have seen that the Primates differ from most other mammalian orders in that there is expressed a tendency towards reduction in number of the thoracicolumbar vertebrae, and that this appears as Rosenberg, Welcker, and others have perceived. The lemurs plainly form the connecting link between Primates and other mammals in this respect as in so many others, and the New-World monkeys stand distinctly closer to the lemurs than to the Old-World apes. The vertebral column of the Old-World apes shows clearly its basic relation to the stem wherefrom successively the gibbons, Man, the giant black anthropoids and the Orang diverged, with concomitant reduction in number of the thoracicolumbar vertebrae. The facts presented here merely serve to confirm these assertions already made. We have carried out the investigation somewhat more searchingly, however, and as a result have found that the number of thoraciolumbars bears an interesting relation to the degree of specialization of the genus. We have been able to follow out this line of thought more particularly among the lemurs and the anthropoids, which groups are in reality most essential for the fundamental accuracy of the general concept. Various suggestive features of the studies by Keith and by Bardeen indicate the correlation between transmutation of the vertebrae and the spinal nerves and plexuses. Hence our next step is the presentation of results of investigation in this laboratory concerning degree of transmutation of vertebrae compared with the constitution of the nerves and plexuses in the same individuals.

#### SUMMARY

1. The following accepted ideas regarding the presacral vertebral column in mammals are undoubtedly correct. The primitive mammalian number of thoracicolumbar vertebrae is and always has been nineteen. In most orders of mammals there is a tendency to increase this number (Welcker's auxispondylous

group). In Primates the tendency is to reduce the number, the anthropoids and Man forming Welcker's lipospondylous group. So far as the thoracicolumbar column is concerned, Man takes his place between the gibbons and the giant black anthropoids.

2. The lemurs form the connecting link between the Primates and other mammalian orders. Whereas some in their specialization exhibit an increase in number like the auxispondylous mammals, there are others which lean towards the typically higher Primates in their tendency towards reduction in number of thoracicolumbar vertebrae. The relation of vertebral formula to specialization in the lemurs is quite significant.

3. The New-World monkeys show a greater tendency towards reduction in number, but here also there is marked bias towards increase in number, as in many lemurs.

4. The primitive number nineteen is characteristic of both subfamilies of Old-World apes, only rare examples showing an increase to twenty.

5. The anthropoids and Man exhibit progressive reduction in number, but of all this group man presents by far the most stable condition numerically in his vertebral formula. The precise stability for Man is strikingly given in the recent work by Willis.

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Resumen por el autor, G. S. Dodds.

El área de las vellosidades coriónicas en  
la placenta a término.

Un niño recién nacido fué objeto de medidas, calculándose después el área de superficie expuesta por las vellosidades coriónicas a la acción de la sangre materna. El método empleado se basa en el estudio de preparaciones microscópicas, calculándose primero el área de las porciones de las vellosidades incluídas en una sección determinada, y con estos datos se calculó su área total para la placenta. El área así determinada mide unos siete metros cuadrados.

Translation by José F. Nonidez  
Cornell Medical College. New York.

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## THE AREA OF THE CHORIONIC VILLI IN THE FULL TERM PLACENTA

G. S. DODDS

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THREE FIGURES

When studying the placenta with classes in embryology, the question has often arisen as to the extent of the surface presented by the chorionic villi to the maternal blood in the intervillous blood space—that is, the surface through which exchange of materials between maternal and fetal blood takes place. If such computations have previously been made, I am not aware of them. No figures of such areas are given in the text-books with which I am familiar, and it is with the hope that the facts may be as new to many other embryologists as to myself that without extensive search of the literature I venture to publish the result of my computations.

As is well known, the placenta consists of the chorion frondosum (fetal part of the placenta) and the decidua basalis (maternal part), a portion of the uterine mucosa. The chorion bears numerous villi which branch extensively in the intervillous blood space which lies between the chorion and the uterine wall. The trunks of some of the attaching or anchoring villi are surrounded by or adherent to the decidua, but by far the greater number, including thousands of small branches, float freely in the blood space, bathed continually with the maternal blood. Through the surface of these villi exchanges of materials take place between the maternal blood in the sinus and the fetal blood which circulates through the villi in the branches of the umbilical arteries and veins.

The following calculations are based upon the study of a single human afterbirth of about average size, one of those in the collection used for demonstration to my classes. On account of

various sources of error to be mentioned later, the figures given are but an approximation, though a very useful one, and I believe no advantage would be gained by the study of more specimens.

The placenta in question (fig. 1) had an outline approximately circular, with a diameter of 16 cm. and an average thickness of about 2.5 cm. The placenta had undoubtedly undergone some shrinkage during the formalin perservation and so is somewhat less in volume than when fresh, but this difference will not so much affect the final result as to greatly lessen its value. The volume, as near as could be computed, was 502 cc., though irregularities of form made such methods rather inaccurate. A more accurate figure, 510 c.c. was obtained by displacement of

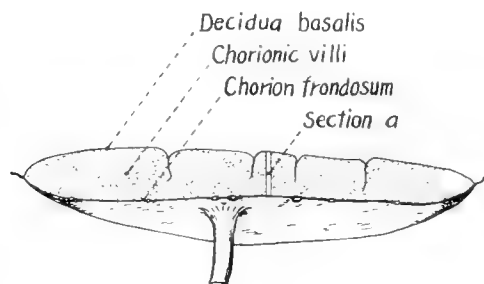


Fig. 1 Placenta cut vertically near its greatest diameter. Position of section from which measurements were made is shown by unshaded band.

water, and has been used the calculations to follow. Of this volume a small part is taken up by the chorion frondosum on one surface and the decidua basalis on the other, with a combined thickness of about 1 mm. (4 per cent of the thickness of the placenta). Making this deduction, about 490 cc. remains as the volume of the blood sinus in which the villi are contained.

#### METHOD

The measurements and calculations are made from microscopic sections as follows: First the parts of villi in a section  $10\ \mu$  thick, extending across the placenta from chorion to decidual surface, were measured and the total surface area of the portion of villi included in this section computed (fig. 1, a). From this was



determined the surface area of the villi in 1 cu. mm. of similar tissue, and from this their surface area in the whole placenta.

The details of procedure are as follows: The whole section including 2,294 sections or slices of villi, was drawn to a known scale by the use of a microprojection lantern. The slices of villi are of various sizes and shapes, as in figure 2, which is a drawing

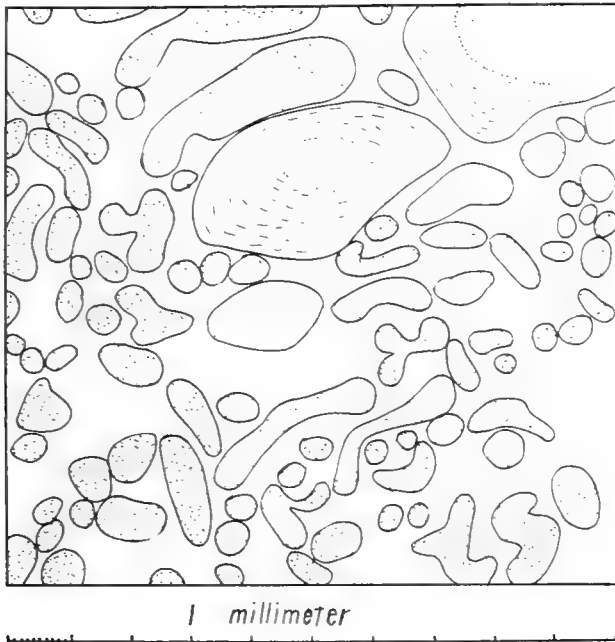


Fig. 2 Part of drawing on which villi were measured, 1 sq.mm. of section included. The rounded stippled areas represent villi cut directly across, the elongated ones those cut diagonally at various angles. The two villi marked partly with line shading are those covered with 'canalized fibrin' and not included in the computed area.

of 1 sq. mm. of the section. The shaded areas represent slices of villi which are of somewhat different sizes, run in all directions, are crooked, and branch richly. The slices of villi in the section would appear, if seen in perspective as shown in Figure 3, *a*, *b*, *c*, which represent sections of cylinders of the same diameter cut at different angles, and when seen from directly above, as under the microscope like *a'*, *b'*, *c'*. The size and shape of the slice

depend not only on the size of the villus, but also on the direction in which it is cut. To compute the surface area of a single slice of a villus is easy, provided it is cut directly across as in figure 3, *a*, where the circumference or perimeter of the slice multiplied by the thickness of the section gives the surface area (the area of the surface shaded with lines in fig. 3, *a*). But when the villus is cut obliquely as in 3, *b* and *c*, the product of the perimeter by the thickness of the section is somewhat less than the actual area, because of the slanting surface at the end of the elliptical sections. In practice, however,

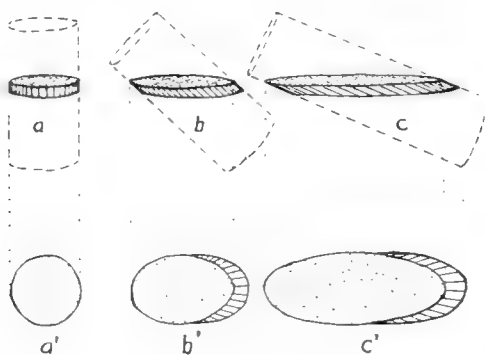


Fig. 3 Diagram to show cylinders of same diameter cut at various angles. *a*, *b*, *c*, showing sections in perspective, *a'*, *b'*, *c'*, projections of these sections, such as the sections of villi as seen in the microscopic section. The part shaded with lines represents the area to be measured, and roughly equals the product of the perimeter of the section by its thickness.

this would probably not be a serious error, because in thin sections, such as actually used, the slanting end shown by the lined surface in figure 3, *b'* and *c'*, is small, and in making the drawing would usually be covered by the width of the line, and so would be within the limit of error of the method employed. We may conclude that the error is not great if we assume that to measure any slice of villus in the section we have but to take the product of the perimeter of the slice by its thickness. This method gives a pretty good approximation of the correct figure, and any constant error is of a negative sign, i.e., the computed area is somewhat less than the actual.

In practice it is not necessary to make separate calculation of the surface area of each villus in the section, but merely to ascertain their combined perimeter and multiply this sum by the thickness of the section and thus obtain the total surface area of all the slices in the section.

The measurements of the drawn slices of villi were made by using a map measurer, an instrument having a small wheel with which to trace the line to be measured, geared to a hand which registers on a dial the distance. Considerable shrinkage takes place during the paraffin imbedding, as shown by measurement before and after. Allowance is made for this in all the linear measurements, and the figures given below are those after this correction has been made.

In making the measurements, care was taken to include only those villi which seemed to have a surface suitable for absorption, that is, those covered with thin, syncytial epithelium, and to exclude those covered with 'canalized fibrin,' these latter mostly of large size, evidently the main trunks from which the smaller ones arise. These smaller ones we will call 'absorbing' villi.

*Measurements of placenta*

Diameter . . . . .	160	mm.
Average thickness . . . . .	25	mm.
Volume by calculation . . . . .	502,000	cu.mm.
Volume by water displacement . . . . .	510,000	cu.mm.
Volume of blood sinus (510,000 less 4 per cent) . . . . .	490,000	cu.mm.
Area of section drawn and measured . . . . .	60.14	sq. mm.
Thickness of section drawn and measured, 10 $\mu$ , or 0.01 mm.		

*Measurements of drawing of section*

Number of absorbing villi in section . . . . .	2,294
Total perimeter of absorbing villi in drawing . . . . .	124,400 mm.
Magnification of drawing . . . . .	155 diam.
Actual perimeter of villi $\frac{124,400}{155}$ . . . . .	802.5 mm.

From these figures one may compute the total surface area of villi in the whole placenta as follows:

Area of absorbing villi in measured section:

$$802.5 \times .01 \text{ mm.} = 8.025 \text{ sq.mm.}$$

Volume of measured section:

$$60.14 \text{ sq.mm.} \times .01 \text{ mm.} = .6014 \text{ cu.mm.}$$

Area of absorbing villi per cubic millimeter of material like that composing the section:

$$\frac{8.025}{.6014} = 13.3438 \text{ sq.mm.}$$

Total area of all absorbing villi in placenta:

$$13.3438 \times 490,000 = 6,538,462 \text{ sq.mm.}$$

$$6.5 \text{ sq.meters}$$

$$69.94 \text{ sq.ft.}$$

This area, as previously pointed out, does not include the total surface exposed within the placenta, but only that of the small villi which, on account of their size and the nature of their surface, seem suited to function as absorbing structures. There is omitted from the above area the surface of those villi, mostly large ones, covered with fibrous or hyaline material, the surface of the decidual septa, and the inner surfaces of decidua basalis and chorion frondosum. From measurements made in the same way as for the smaller villi, it appears that these surfaces include nearly  $\frac{1}{10}$  of the total internal surface of this placenta, which thus is about 7 square meters.

The functional surface of the absorbing villi is doubtless reduced somewhat by the numerous thickenings in their epithelium in the form of masses of nuclei, variously known as 'proliferation islands,' 'nuclear groups,' etc., and by the crowding of villi, so that surfaces of adjacent villi are in contact and thus not freely exposed to the maternal blood. Thus it appears that the effective, absorbing surface within the placenta includes not the total of 7 square meters, nor the measured area of the absorbing villi, 6.5 square meters, but a figure somewhat less than this and hard to estimate.

Inasmuch as the above calculations are based upon measurements of but one section with an area of 60.14 sq.mm., sections

from other parts of the placenta were studied, the number of villi per sq.mm. counted and measures of the size of these villi taken. From these observations it seems that the section which forms the basis of these figures is representative, and no great difference would appear if several sections were measured. That is, in any section of this size, no matter from what part of the placenta it is cut, we find about the same number of villi per sq.mm. and the average size of the villi cut is remarkably constant.

Concerning the method used, it is to be noted that though the actual microscopic section was cut  $10\ \mu$  thick, the result would have been the same had it been  $5$  or  $15\ \mu$  or any other thickness, because the slices of villi in the section are assumed to be cross-sections of various shaped bodies extending in a direction perpendicular to the plane of the section not only within the section, but indefinitely outside the section to the border of the placenta. Not only this, but all the villi of the placenta are assumed to be straight, parallel bodies of the same average size as those measured. The element of truth in this assumption is that in any section cut, there appear as many villi, and of the same size, as in the one measured, though not the same villi. The element of error is the oblique direction taken by many of the villi in the section, which makes their actual area less than the computed area.

The result of 6.5 square meters (70 sq.ft.) is so large as at first to seem unreasonable. In order to try to surprise myself into inconsistencies, I tried several modifications of the method and always arrived at the same result. Certain figures from such calculations may be of interest: the average number of villi cut per sq. mm. was 38, in some areas running to a number above 100, in others much fewer on account of less dense arrangement and presence of large trunks of villi. In a complete vertical section across the placenta there would be cut 133,760 villi, and in a complete horizontal section 763,648. The average perimeter of the sections of absorbing villi measured is 0.357 mm.—a figure from which there is a surprisingly small deviation when any area as large as 1 sq.mm. is measured. A villus of

this average size and with a surface area of 6.5 square meters would have a length of a little more than 18 kilometers ( $11\frac{1}{4}$  miles). Lest this measure may seem excessive, we note that a cylinder of this length and area will have a diameter of 0.113 mm., and occupies a volume of 174,000 cu.mm. (174 cc.), about 37 per cent of the volume of the blood sinus in which the villi actually float, leaving 63 per cent of the space for the circulation of the maternal blood—a relation which, from a general inspection of the sections, seems not unreasonable.

As a matter of fact, the diameter of this 'average' villus (0.113 mm.) is larger than the actual free terminal branches of the absorbing villi, because many of these sections are oblique, and also a few of the larger trunks are included in the measured sections. The diameters of these free villi, as actually measured both on an enlarged drawing and on the sections, fall mostly between 0.03 and 0.06 mm., so that the combined length of the villi must be considerably in excess, possibly nearly double the figure, 18 kilometers, given above.

#### SUMMARY

The total surface area of the chorionic villi exposed within the placenta described above measured about 7 square meters, of which 6.5 square meters was included in those numerous small branches which by nature of their surface seem suited to act as organs of absorption. The efficiency of these is doubtless further reduced by the numerous thickenings of their epithelium in the form of the masses of nuclei so abundant on the surface of these villi.



Resumen por la autora, Alice L. Brown.

Un simple aparato para la microinyección a mano.

El presente trabajo es una descripción de la construcción y funcionamiento de un aparato de microinyección a mano, basado en un aparato semejante descrito recientemente por Chambers en esta revista. Este aparato, de fácil construcción, puede emplearse para inyecciones en frío o en caliente. Asegura gran delicadeza y libertad de movimientos, de tal modo que los vasos sanguíneos de embriones muy jóvenes pueden fácilmente inyectarse.

Translation by José F. Nonidez  
Cornell Medical College, New York.



## A SIMPLE APPARATUS FOR DELICATE INJECTIONS.

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ONE FIGURE

Methods and apparatus for dissecting and injecting cells have been worked upon for a number of years. With the micromanipulator apparatus recently described by Chambers<sup>1</sup> is an injection device consisting of a syringe, a brass tube, and a glass cannula. This device, which is a very simple one, may be easily adapted to injecting the blood vessels of chick embryos or any delicate accurate free-hand injection work under a binocular dissecting microscope. The accompanying illustration shows this device held in a system of ball-and-socket joints which takes the place of the micromanipulator. In addition is a scheme providing for a warm injection mass.

For descriptive purposes this apparatus is divided into three parts:

First, the actual injecting part consists of a syringe<sup>2</sup> (*A*); a hypodermic needle with the tip sealed into one end of a coiled piece of fine extra soft brass tubing<sup>3</sup> (*C*); and into the other end of which (*n*) is sealed a piece of  $\frac{1}{8}$  inch glass tubing (*D*). The glass tube (*D*) is drawn out at one end (*p*) to about 1 mm. inside

<sup>1</sup> Chambers, R. New apparatus and methods for the dissection and injection of living cells. *Anat. Rec.*, vol. 24, 1922.

<sup>2</sup> A 2-c.c. Luer syringe is shown in the figure, but any convenient syringe may be used.

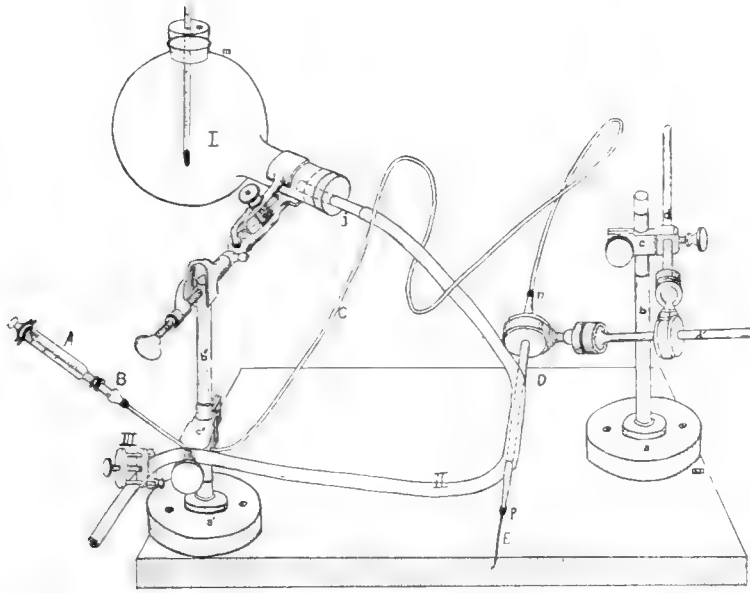
<sup>3</sup> The soft brass tubing used here is one with 2 mm. outside diameter. This or others of somewhat larger sizes may be obtained from any wholesale brass dealer or automobile supply house. If the tubing is too stiff, it may be softened by annealing.

diameter, into which the cannula (*E*) is sealed.<sup>4</sup> The cannula is made by drawing out a glass capillary which will fit into the drawn out end of *D* at *p*. The tip of the cannula is made by momentarily heating the capillary over a microburner and pulling it out just as it softens. This procedure results in a tapering tip which may be broken off to form a pipette of the size which suits the operator. The cannula may be bent at any desired spot to facilitate insertion into the tissue to be injected.

Second, the support for the injecting device in the figure is built out of two Leitz dissecting stands. One complete stand, and the ball-bearing arm, *d'*, of a second, connected together with wooden plugs, as shown in the figure, gives ample freedom for moving the glass tube (*D*) which bears the injecting cannula (*E*). The coils of the brass wire allows the cannula to be moved about while the syringe is held rigid by the clamp, *c'*.

Third, if a warm injection be desired, this is accomplished by arranging a flow of warm water to heat the injection fluid in the glass tube (*D*). This is done by means of a 500-cc. flask (*I*) fastened on the support (*b'*) and a  $\frac{1}{4}$  inch rubber tube connected with it at *j*. The glass tube (*D*) is thrust through the wall of the tubing in two places as indicated in the figure. The lower end of the rubber tube is shunted off to one side of the apparatus and the outflow of the warm water is regulated by the screw clamp (*III*). The water in the flask (*I*) can be heated by an alcohol lamp or a small Bunsen burner and its temperature regulated with the aid of a thermometer. Through the cork (*m*) a small funnel may be inserted for the addition of more water. If a flask with an opening as shown in the figure is not available, a second glass tube must be thrust through the cork of the flask (*I*) to allow the entrance of air. A third tube may be added for the addition of water.

<sup>4</sup> The sealing may be done with ordinary sealing-wax or de Knotinsky cement, which can be obtained from any dealer in chemical and physical apparatus. As the micropipette has to be frequently changed, it must be sealed with a cement which can be readily softened. The de Knotinsky cement is admirable for this purpose.



Once this apparatus is set up, the bases *a* and *a'* may be screwed down to a board of convenient size, thus making a permanent apparatus of which only the cannula has to be renewed.

To operate the apparatus the brass tube (*C*) must be filled with water through the syringe before the cannula (*E*) is sealed at *p*. The injection mass is drawn up through the cannula, an air space being left between the water and injection mass to prevent diffusion. The tissue or embryo to be injected is placed in position under a binocular dissecting microscope, and the injecting device brought into position with the cannula immediately over the tissue. The operator's fingers control the cannula by gripping *D* where the rubber tubing covers it.<sup>5</sup>

<sup>5</sup> For other types of apparatus for similar purposes, see Knower, H. McE., A new and sensitive method of injecting the vessels of small embryos, etc., under the microscope. *Anat. Rec.* vol. 2, no. 5.

Resumen por el autor, Morie F. Weymann.

El comienzo y desarrollo de la función en la  
médula suprarrenal de los embriones de cerdo.

Puesto que los experimentos microquímicos y los realizados en tubos de ensayo indican que el precipitado moreno formado cuando se añade epinefrina al bicromato potásico es el mismo material que los gránulos morenos formados en la médula de las glándulas suprarrenales fijadas en soluciones que contienen bicromato potásico, la aparición de gránulos (la reacción cromafínica) en las suprarrenales convenientemente preparadas puede emplearse como un indicador de la presencia de la secreción. Las suprarrenales de los embriones de cerdo, preparadas con este propósito, han sido estudiadas por el autor, quien ha llegado a las siguientes conclusiones: 1) La adquisición de la función secretora comienza gradualmente, comenzando en el estado de 40 a 45 mm. y aumentando hasta el estado de 142 mm., después del cual la reacción permanece sensiblemente constante. 2) Con el comienzo de la función tienen lugar cambios evidentes en el citoplasma y núcleo de las células activas. 3) Algunas células comienzan a presentar la reacción cuando aún están fuera de la corteza y a considerable distancia de su posición definitiva. 4) No todas las células comienzan a funcionar al mismo tiempo. 5) La división celular tiene lugar en ciertas células que presentan una reacción cromafínica definida.

Translation by José F. Nonidez  
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## THE BEGINNING AND DEVELOPMENT OF FUNCTION IN THE SUPRARENAL MEDULLA OF PIG EMBRYOS

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FOUR FIGURES

Since the beginning of intensive study of the endocrine glands, these organs have held an important place in the interest of students of growth and differentiation. It has been amply demonstrated that among some of the lower vertebrates endocrine function plays a significant rôle in the regulation of development and metamorphosis, and there are indications that in the mammals it is no less important. In the case of man, it has been suggested that variation in physical type is largely dependent on variation in endocrine activity during growth. What part the glands of internal secretion play in regulating development will probably have to be determined largely by experimentation, but before conclusive experiments can be carried out it will be necessary to have a knowledge of the normal function of the endocrine glands during the embryonic period. A search of the literature shows that a number of attempts have been made to determine whether or not these glands function in the embryo, and that considerable evidence has accumulated on this point. The present work was undertaken with the view of making a study of a single gland in some convenient form in order to determine, if possible, when and how it normally begins to function.

On reviewing what is known of the development of the endocrine glands and considering the feasibility of available tests for function, the suprarenal medulla was selected as best suited for an initial study of this kind. The pig, while not well suited for experimentation, was nevertheless selected because of the abundance of easily available material and the completeness of our knowledge of its development.

Other investigators have commonly relied on biological and chemical tests of the extract from embryonic suprarenals in their attempts to determine the presence or absence of function in these glands. Lewis ('16) states that Moore and Purinton were unable to find evidence of adrenalin in the foetal suprarenals of man by the  $\text{FeCl}_3$  and blood-pressure tests. Svehla ('00) also reported similar results with human material, but he did find adrenalin present in glands from foetal dogs, as did Langlois and Rehns ('99) in those from sheep foetuses. Lewis was unable by chemical and biological tests to demonstrate it in human foetal suprarenals, but by the uterine strip test he got positive indications of its presence in extracts from the suprarenal gland of two full-term still-born infants. Lewis also tried out the color test of Folin and Denis, but concluded that it is useless in the study of foetal suprarenals, because foetal tissues in general are rich in uric acid, which gives the same blue color reaction as epinephrin.

Fenger ('12) found epinephrin by the iodic-acid method in the extract of suprarenals from pig embryos of seventy days; also in that from beef embryos of three months, and sheep embryos of three to four months. McCord ('15) showed, by the uterine-strip method, evidence of epinephrin in the suprarenals of beef embryos as early as the 113-mm. stage. Biological tests could not be carried out on younger embryos because the suprarenals were not large enough to furnish sufficient extract, even when many were used. Langlois and Rehns believed they were able to demonstrate an effect of the suprarenal extract from sixty-day sheep embryos upon the blood pressure of a dog.

Thus it may be seen that there is considerable evidence in the literature that the foetal suprarenals do function, and that they begin to function at an early period. But since there are some possible uncertainties with the biological tests, it seems desirable that the results obtained by them should be checked and supplemented by a thoroughly reliable microchemical test. In the chrome reaction of the medullary cells, discovered by Henle ('65) and interpreted chemically by Ogata and Ogata ('17), we seem to have at our disposal just such a test. Since the time of

Henle it has been known that suprarenals fixed in dichromate solution show a yellow-brown color in the cytoplasm of the medullary cells. This was for a time thought to be a complex adrenalin chromate compound. But the recent work of Ogata and Ogata has furnished convincing evidence that it is a simple inorganic salt, chromium dioxide. It is formed by the reducing action of adrenalin on the dichromate. Kingsbury ('11) has presented evidence pointing in the same direction. He finds that the precipitates formed by various fixing fluids acting on the fresh suprarenal medulla are identical with those produced by the same fluids acting in vitro on adrenalin.

The most critical of the Ogatas' experiments have been repeated and corroborated by the writer. It is found that if commercial adrenalin is added to Müller's fluid in a test-tube, a yellow-brown precipitate slowly forms. Under the microscope it has the same color as is revealed in the cytoplasm of the medullary chromaffin<sup>1</sup> cells after fixation in Müller's fluid. Chemical analysis of this precipitate shows it to be  $\text{CrO}_2$ . As the precipitate is very soluble in alkali, and to a less degree in mineral acids, it was thought advisable to see if these reagents would affect it in the same way in an albuminous solution as in the chromaffin cells themselves. To test this point, a series of sections from a suprarenal gland fixed in Müller's fluid was mounted on slides in the usual way, while on another set of slides was placed a thin

NO.	TREATMENT	RESULT	
		TISSUE	PRECIPITATE
1	Haematoxylin and eosin stain	No change	No change
2	H. and e. stain with ammonia water	No change	No change
3	H. and e. with $\text{NH}_4$ and also acid alcohol	No change	No change
4	H. and e. with 2N HCl for 10 min.	No change	Partially dissolved
5	H. and e. with 2N NaOH for 3 min.	Partially dissolved	Partially dissolved
6	H. and e. with 2N NaOH for 10 min.	Partially dissolved	Partially dissolved
7	H. and e. with 2N NaOH for 15 min.	Dissolved	Dissolved

<sup>1</sup> The term chromaffin seems to have been first applied to cells which show the Henle reaction by Kohn ('98).

coating of the precipitate suspended in albumen fixative. The slides were numbered, and one of each set subjected to each of the several treatments indicated in the accompanying table.

Thus it is seen that the precipitate on the slide surrounded by coagulated albumen behaves in much the same manner with regard to solvents as that in the chromaffin cells. The fact that the acid did not affect the granules in the tissue as much as those in the artificial coagulum may very probably be explained by the more intimate relation of the precipitate to the natural protoplasm than to this artificially prepared albumen mixture. These supplementary experiments tend to support the evidence of the Ogatas that the chromaffin substance which appears in the cells is identical with the precipitate that is produced by adrenalin in the test-tube. Therefore the appearance of the Henle reaction in the suprarenal medulla has been considered by the writer as coincident with the appearance of epinephrin in this gland.

Prentiss and Arey's Textbook of Embryology contains the statement that the chrome reaction is an indication of the beginning of function of the gland. The data on which this statement is based are not mentioned in the book, and the writer has not been able to find a specific statement to this effect elsewhere in the literature.

The most satisfactory data with reference to the chrome reaction was found in an article by Wiesel ('01) on the development of the suprarenal medulla. Here it is stated, under his description of a 51-mm. pig, that "die in Chromsalzen geharteten Organe dieses Stadiums geben noch keine chromaffine Reaktion," which would seem to indicate that his next stage, a 63-mm. embryo, showed the brown color. In the description of the 63-mm. size he makes no mention of chrome reaction. If the stage at which Wiesel first found the reaction were at 63 mm., then one might infer that the assumption of function occurs between 51 and 63 mm. But the writer finds that the reaction occurs in stages younger than these. This fact may have been easily overlooked by one not particularly interested in this phase of the problem.

In the series studied here embryos of 218, 142, 85, 75, 65, 55, 45, 40, 30 and 24 mm. were used. Other sizes were studied, but



the above suffice to show the steps in the assumption of function by the suprarenal medulla. There were always several individuals of each size sectioned to check the results, and, when preparing glands from smaller embryos, an older suprarenal of a stage known to show the chrome reaction was always run through in the same container, in order to preclude the possibility of drawing erroneous conclusions on the basis of fortuitous variation in technique. The most satisfactory chrome salt for fixing was found to be the potassium dichromate in Müller's fluid. In the larger embryos the suprarenals were dissected out, the small embryos were sectioned in toto.

The method was as follows: After being fixed in Müller's fluid for two days, the specimens were washed in running water for one hour and then hardened in 70 per cent alcohol for two days more. They were then dehydrated, sectioned by the paraffin method, and stained lightly with neutral haematoxylin and eosin. In the younger stages some were stained only with haematoxylin in the hope of finding fainter chrome reactions, but this did not help as the faintest reaction could be distinguished over a light eosin stain. Washing the specimens for twenty four hours was also tried to see if the precipitate could be washed or dissolved out in that time, but there seemed to be no tendency for this to occur.

In the following account of the suprarenal of the pig at different ages no attempt is made to trace the development of the gland as a whole or to enter into the details of its histology, attention being concentrated on the appearance and development of the chromaffin reaction.

*24-mm. stage.* There is an accumulation of cells, apparently all of the same type, medial to the Wolffian body in the position of the future suprarenal gland. These cells, which are rather polyhedral, with no special cytoplasmic differentiation and round nuclei, have a tendency to be arranged in cords with open spaces between them. Their subsequent history shows them to be cortical cells. There is no evidence of medullary cells in the gland at this time. In the sympathetic ganglion region is a

rather large group of cells which are quite embryonic in type and give no trace of a chromaffin reaction.

*30-mm. stage.* At this stage cells similar in appearance to those in the sympathetic ganglion are seen in the mesenchyme between the site of the suprarenal medulla and the ganglion. There is no brown color in their cytoplasm and the nuclei are rather large and loosely reticular. A few small clumps of these cells appear on the periphery of the suprarenal anlage. The appearance would lead one to suspect migration from the ganglion.

*40-mm. stage.* Beginning with this stage, the suprarenals were dissected out with a liberal amount of surrounding tissues, but the sympathetic ganglion was not included. Cells resembling those of the ganglion extend from its direction toward the gland. Several groups on the periphery of the suprarenal anlage appear to be penetrating between the cortical cells, and there is a difference in structure of the cells in any one group, some tending to become further differentiated as indicated by a decrease in size of the nucleus, which at this time becomes more compact and more deeply staining. There is the faintest suggestion of a chrome reaction, that is, a yellow-brown color, in the cytoplasm of these latter cells.

*45-mm. stage.* In this stage a few of the cells which seem to come from the sympathetic system are inside the cortical mass and numerous groups are on the periphery, with others scattered along toward the sympathetic ganglion region (fig. 1). In the cells with the darker, more deeply staining nuclei there is a faint, but definite, yellow-brown color in the cytoplasm (fig. 2). This color cannot be observed in mesenchymal or renal cells in the neighborhood. Some of these chromaffin cells are found in groups which are at some distance from the cortical anlage in the direction of the sympathetic ganglion. As these clumps of cells increase in size and as mitotic figures (fig. 3) are seen among them, the assumption is justified that they are multiplying as well as maturing.

*55-mm. stage.* Groups of medullary cells are evident in the periphery and toward the center of the cortical mass. About half of the medullary cells show the yellow-brown chrome re-

action, which is of a somewhat deeper color than in those of the 45-mm. stage. The color also varies in the individual cells within a group, being apparently deeper in those which, judged by their nuclei, are most differentiated.

*60 mm. stage.* There is little change from the 55-mm. stage except that the chrome reaction is more marked and more general, and some groups of medullary cells are situated more deeply in the cortical mass.

*65-mm. stage.* The reaction is present in the majority of medullary cells. Islands of these latter are scattered throughout the cortex. The relative amount of medulla is less than in later stages. Mitotic figures are seen in certain chromaffin cells.

*75-mm. stage.* Chromaffin cells are for the most part in the middle portion of the gland cortex, with some peripheral and some central. The reaction is more marked, and is now of about the same intensity in all the medullary cells.

*85-mm. stage.* In this stage the medulla is not yet entirely central. All medullary cells show a definite chromaffin reaction, but the maximum intensity has not yet been reached. Those on the periphery are as dark as those which are central in position. The nuclei of the chromaffin cells are not so dense as in the earlier stages, but do not have the loose network characteristic of the primitive undifferentiated cells of stages before the first appearance of the chromaffin reaction.

*142-mm. stage.* All medullary cells are central except one group seen on the periphery of the gland and a few strands running into the cortex. The intensity of the reaction in the individual cell has now reached its maximum (fig. 4) and is not darker in older stages.

*218-mm. stage.* Chromaffin cells are all centrally placed constituting a typical suprarenal medulla. The density of color is the same as in the 142-mm. stage. The individual cells have fairly abundant cytoplasm with rather dark-staining granules and spherical or oval nuclei. Many of the nuclei appear to have a brown tint, but careful study of the preparations shows that when this appearance is observed it is to be attributed wholly to

the brown granules in the overlying cytoplasm. The cortical and tissue cells show no brown color in the cytoplasm.

The observations recorded in the preceding descriptions may now be recapitulated in a few paragraphs which, it may be recalled, refer only to suprarenals of pig embryos that have been treated according to the technique indicated at the beginning of this paper.

1. The earliest indication of the chromaffin reaction that can be detected appears in embryos of about 40 mm. In embryos of 45 mm. the reaction has become very definite, from which fact it appears that it is at about this time that medullary cells begin to produce, or at least to store, adrenalin.

2. In early stages the reaction is observed in cells which are not at the site of the future suprarenal gland. This observation presents no difficulty if one accept the view that the medullary cells reach their definitive position by migration from the sympathetic—a view which is clearly set forth by Whitehead ('02) and by Wiesel, who states that there is no longer any doubt that the suprarenal medulla "*stammt einzig und allein vom Sympathicus und dessen Ganglien*" (loc. cit., p. 141).

3. Assumption of the ability to give the chromaffin reaction occurs concomitantly with modification in the histological appearance of the cell, involving changes in the size and staining reaction of the nuclei. The suspicion might have arisen that these changes, including the chromaffin reaction, indicate degeneration of certain cells in each group, but no evidence was found that such is really the case.

4. Since cells that show the reaction are found in process of mitosis, it is clear that the function begins in a relatively undifferentiated condition of the cells, which are both multiplying and specializing as they approach their definitive position.

5. The fact that in the intermediate stages cells in the medullary region and those at the periphery of the cortex may show the same color intensity gives further evidence that the function of a cell is not wholly dependent on its position. The more mature cells, as indicated by the chromaffin reaction, are not necessarily the first to reach their destination.

6. The assumption of the function which is indicated by a chromaffin reaction takes place gradually in the medullary cells of embryos from 40 mm. to 75 mm. in length. After the latter stage apparently all the medullary cells show the reaction. Its intensity in individual cells increases from the 40-mm. to the 142-mm. stage.

In summary, the development of function by the medullary cells of the suprarenal gland may be briefly stated as follows: The groups of embryonic cells found in the 24-mm. embryo between the sympathetic ganglia and the anlage of the suprarenal cortex give no indication of specific function. At between 40 and 45 mm. certain of them begin to show a faint chromaffin reaction in their cytoplasm, their nuclei at the same time becoming denser, smaller, and more deeply staining. As the embryo grows these medullary cell groups penetrate the cortical anlage and finally occupy its central portion at the 142-mm. stage. During this process mitosis continues, all the cells gradually assume the capacity for a darker constant chromaffin reaction, the relative amount of cytoplasm increases, and the cells become from one and one-half to two times their original size. The nuclei are no longer as dense as when the cells first began to exhibit the chromaffin reaction, but are smaller, darker, and more granular than in the undifferentiated cell.

These findings tend to confirm and supplement the results, so far as they go, of other workers who have used different methods and material. They fix more closely than has been done before the time at which one of the mammalian endocrine glands begins to function in a definite manner, and show that not all the medullary cells of the suprarenal gland begin their activity at the same time or in the same place. Of especial interest is the indication that the functioning of the medullary cells is dependent upon some intrinsic factor in the cell itself rather than upon its position with reference to other tissues. Since no evidence of medullary function was found in embryos under 40 mm., at which time the principal organs and systems are well established, it does not seem probable that any primary

malformations can be traced to disturbances in the epinephrin production of the embryo itself.

The writer is indebted to Dr. C. H. Danforth for suggesting the problem and for criticism of the work.

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## PLATES

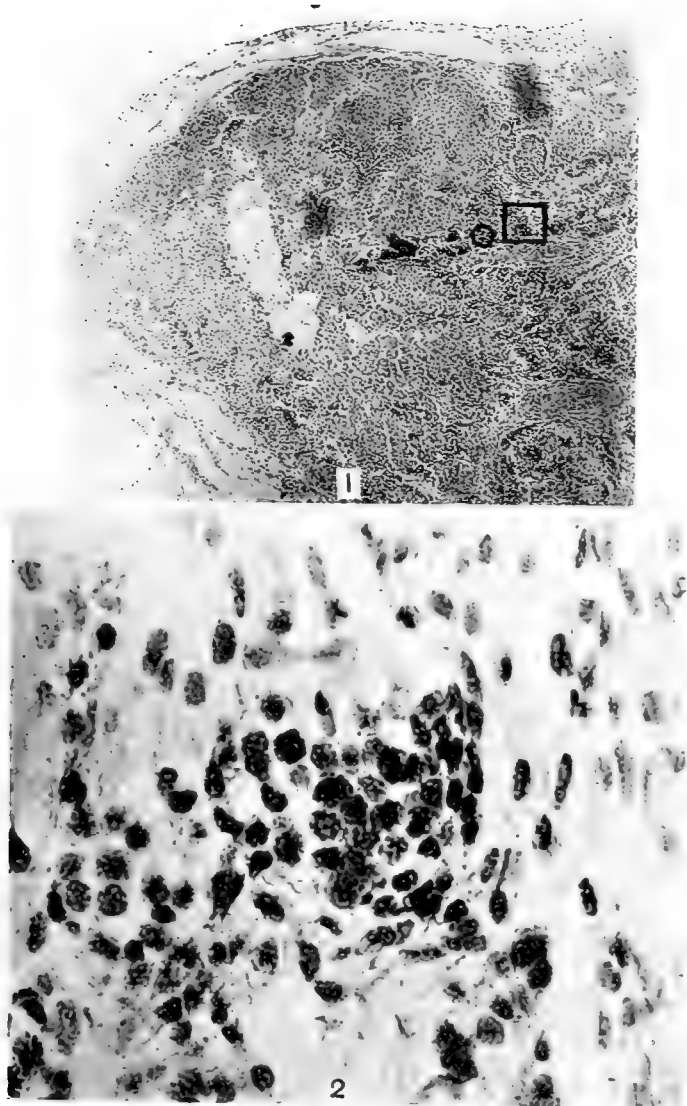
## PLATE I

### EXPLANATION OF FIGURES

1 Section of the suprarenal gland from a 45-mm. pig embryo fixed in Müller's fluid and stained with neutral haematoxylin and eosin. Magnified about  $\times 40$ . The specimen shows, to the right, strands of chromaffin cells at and near the hilus and to the left the cortical part of the gland. The square indicates the region shown in figure 2, the circle that shown in figure 3.

2 Higher magnification of the region marked by a square in figure 1. The darker cells with small dense nuclei are the ones in which the chrome reaction is first definitely evident. The embryonic character of the other cells may be noted. This group of cells while near the cortex is not surrounded by it. Magnification about  $\times 700$ .



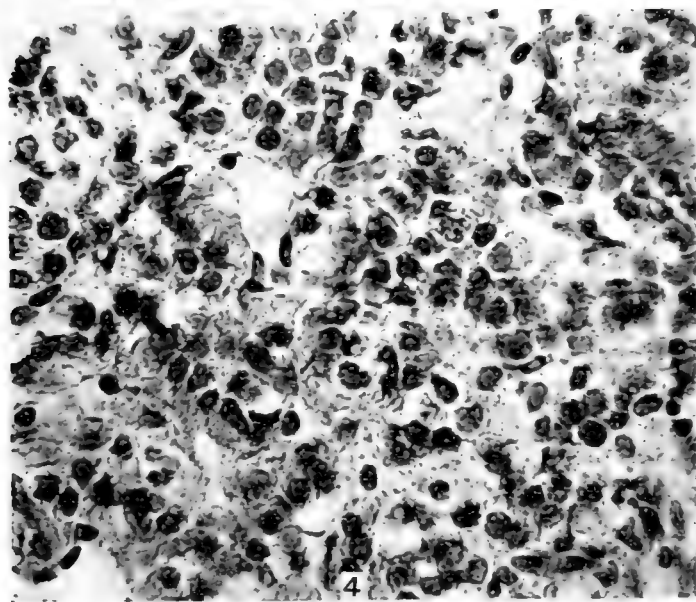
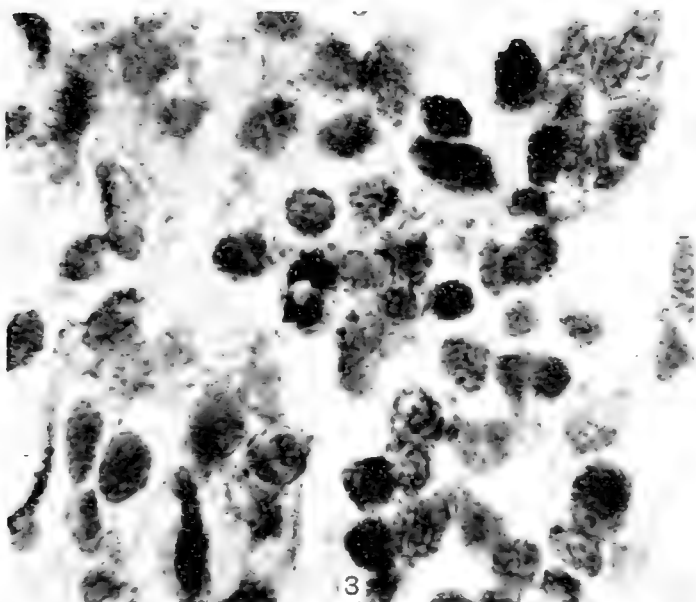


## PLATE 2

### EXPLANATION OF FIGURES

3 Area within the circle marked on figure 1, selected to show mitosis in a cell which already exhibits the chromaffin reaction. Magnification about  $\times 960$ .

4 A region of the suprarenal gland of a 142-mm pig embryo fixed in Müller's fluid and stained with neutral haematoxylin and eosin. In this section the medullary cells show the chromaffin reaction at its height. The granules in the cytoplasm are of a yellowish brown color. Magnification about  $\times 700$ .



Resumen por los autores, H. B. Goodrich y J. A. Scott.

El efecto de la luz sobre los cultivos de tejidos.

El presente trabajo describe los resultados de experimentos llevados a cabo con el fin de comprobar el crecimiento relativo de los cultivos de tejidos a la luz y en la oscuridad. El material empleado ha consistido en trozos del corazón del embrión de pollo. Los autores han empleado una luz más intensa que la usada ordinariamente para las observaciones microscópicas o que la empleada para calentar las incubadoras. Los resultados obtenidos indican que los tejidos durante el crecimiento inicial desde el trozo cultivado prosperan del mismo modo en la luz que en la oscuridad. Los autores describen el aparato empleado para incubar los tejidos a iguales temperaturas a la luz y en la oscuridad.

Translation by José F. Nonidez  
Cornell Medical College, New York.

## THE EFFECT OF LIGHT ON TISSUE CULTURES

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### ONE FIGURE

The following experiments were designed to test the effects of ordinary types of illumination on tissue cultures. In the course of other work with tissue cultures, the question had arisen as to whether the method of heating an incubator with an electric-light bulb would prove harmful. Also C. C. Macklin ('16) has reported that ordinary illumination for microscopic observation caused degeneration of cultures, while on the other hand S. J. Holmes ('14) has stated that "Light has very little effect on epithelial cells in tissue cultures."

The incubator used was one which had been made in the University workshops and which is heated by electric resistance wires giving no light. It was necessary to have a control series of cultures kept in the dark at the same temperature as those exposed to light. The apparatus used to maintain these conditions for the two sets of cultures is illustrated in figure 1, and consisted of the following parts. Two cubical tin cracker boxes having hinged doors and measuring about 10 inches on a side were placed on the upper shelf of the incubator. One of these was the dark box (*H*) and the other the light box (*I*). Light was admitted to the incubator and the light box through windows. The source of light was a 600-watt electric lamp, with tungsten filament, filled with inert gases, and commercially known as Mazda, type C, stereopticon tubular bulb, style T, 20. This was placed (at *A*) in a stereopticon; the projection lenses were removed, but the condensing lenses (*B*) retained. The light passed through the condensing lenses and next through the circulating water-bath (*C*), which was originally a part of another projection apparatus. The light was then reflected by a mirror

(*D*) placed at an angle of  $45^{\circ}$  to the vertical, and passed successively through the projection lenses (*E*) of the stereopticon, a crystallization dish containing water (*F*), a window in the roof of the incubator, a window in the light box closed by a glass plate (*G*), and then on to the tissue cultures placed on a shelf in the light box.

This method proved adequate for the removal of all heat radiation, and thus the temperature of the two boxes remained very nearly equal. About five observations of temperature were made each day for both light and dark boxes. Usually the same temperature was noted in both readings, and there was seldom a difference greater than  $1^{\circ}$  C. Temperatures were maintained within the usual range for chick tissue incubation  $-37$  to  $39^{\circ}$  C.

The light reaching the cultures was measured by a luxometer which had been previously calibrated by the United States Bureau of Standards. The measurements showed a light intensity of about 270 foot-candles with a possible error of 20 per cent. This is about equal to the light yielded by a tungsten bulb of 200 watts placed 10 inches from the illuminated surface, and thus is greatly in excess of light from bulbs used as a source of heat in incubators. Further, this intensity of light was compared with that used in ordinary microscopic illumination. The amount of light from a microscope lamp with 'Daylite' glass placed immediately in front of the microscope mirror and with diaphragm open wide was compared with a light of known intensity.<sup>1</sup> It proved to yield 158 foot candles. This was a greater intensity of light than that which had ordinarily been used in the observation of cultures, as the lower diaphragm was usually partially closed. It was, however, considerably less than the maximum illumination obtainable by placing the light directly under the microscope and with the diaphragm wide open. This was measured to give 1099 foot-candles.

Cultures were made of heart tissue of chick embryos of from seven to fifteen days' incubation, and these were planted in the usual manner in hanging drops on the under surface of coverslips sealed over depression slides. Two media were used: in

six experiments the blood plasma of the fowl, and in three experiments the sea-water and chicken bouillon as used by M. R. Lewis ('14). Tissue from the same heart was used in each ex-

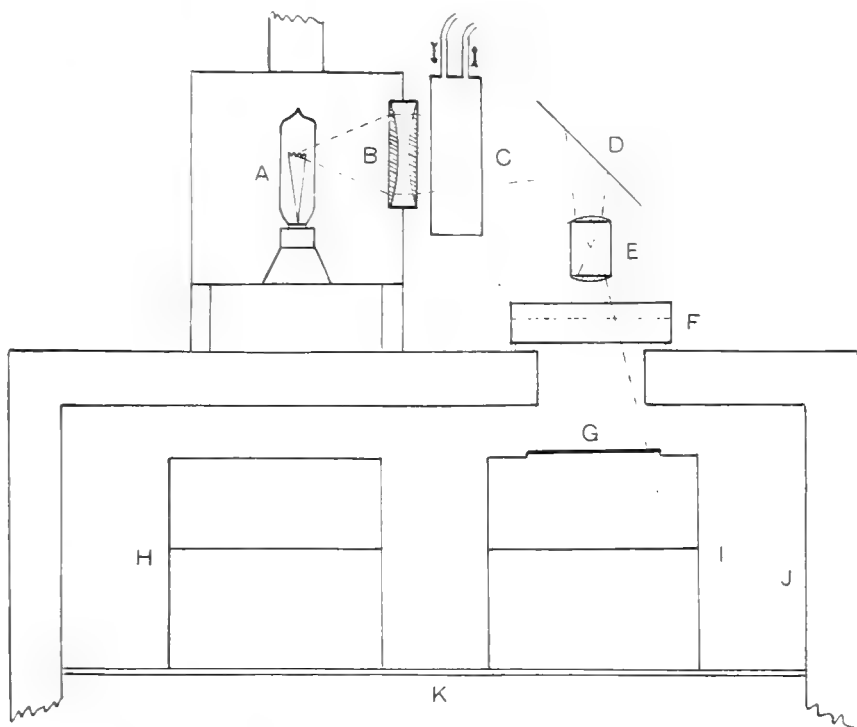


Fig. 1 Apparatus for incubating tissues at equal temperatures in light and dark. *A*, electric lamp; *B*, condensing lenses; *C*, circulating water-bath; *D*, mirror; *E*, projecting lenses; *F*, water-bath; *G*, glass window; *H*, dark box with shelf for cultures; *I*, similar light box; *J*, wall of incubator; *K*, shelf of incubator.

periment for both sets of cultures. Usually not more than eight or ten cultures could be placed in the lighted area. The results of one experiment, as an example, and the total of all experiments are shown in tables 1 and 2, respectively. The designation of slight, moderate, and abundant growth is arbitrary. The term growth is used as has been the practice in tissue-culture work to

indicate the migration of cells from the explant without definite knowledge of the amount of cell division involved. 'Slight' growth indicates a condition where only a few isolated strands of cells were observed. 'Moderate' growth means a condition of growth more vigorous than slight growth up to a condition such that the explant is surrounded by cells extending a distance about equal to one-half of its diameter. And finally, 'abundant' growth signifies further spreading, which was seldom greater than a distance equal to the diameter of the explant. The observations of the conditions of the cultures were made up to five days after planting, by which time the active wandering of cells had usually ceased in all cultures. The average period of growth upon which observations in the tables are based was four days. Muscular contractions were frequently noted in both series of cultures. In a few cases stained preparations showed mitotic figures in wandering cells. These were noted in both types of cultures, but were so infrequent as to render comparative data of little value.

It will be noted from these tables that growth in the light box was practically as good as that in the dark box, and the variation probably lies within the range of variation due to other causes. There are certain probable causes of variation in tissue cultures which are difficult to obviate. These are variations in size of drop of medium, in size of the explant, in period of evaporation of medium during process of mounting.

These experiments seem clearly to indicate that polychromatic light from an incandescent electric bulb of not over 270 foot-candles has no deleterious effect in the initial growth of tissue cultures from the heart of the chick embryo. Thus the illumination used in incubators and in ordinary microscopic observations is harmless.

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TABLE 1

*Experiment started May 7, 1921. Cultures grown for five days*

	NO GROWTH	SLIGHT GROWTH	MODERATE GROWTH	ABUNDANT GROWTH
In light	2	0	4	4
In dark	1	0	7	2

TABLE 2

*Summary of all experiments*

	NO GROWTH	SLIGHT GROWTH	MODERATE GROWTH	ABUNDANT GROWTH	TOTAL
In light	14	11	19	24	68
In dark	14	15	19	16	64

<sup>1</sup> Estimate of light intensity was made by comparison with light of known power by the greased-spot method. A light of 247 candle-power gave at a distance of 15 inches illumination equal to that from the microscope lamp focused by a condenser at the position of a microscope slide. The following formula was used for conversion into foot-candles:  $\frac{c \sin \phi}{d^2}$ .  $c$  is the known

candle power,  $\phi$  the angle between light rays and surface, in this case  $90^\circ$ , and the  $\sin$  is 1.  $d$  is the distance in feet from the illuminated surface. By substitutions the formula then reads:  $\frac{247 \times 1}{(\frac{5}{4})^2} = 158$ .

Resumen por el autor, A. B. Dawson.

El origen y presencia de una sola arteria umbilical  
en los fetos humanos normales y anormales.

En la literatura se han descrito dos grupos de arterias umbilicales impares. En el primer grupo se incluyen aquellas que se originan en la aorta; en el segundo las que se originan en el tronco de la iliaco-hipogástrica común. En el presente trabajo se describen dos casos representativos de estos dos grupos. El perteneciente al grupo primero fué hallado en un mónstruo simpódico, el otro aparecía en un feto hembra formado normalmente. Las arterias umbilicales impares que se originan en la aorta están generalmente asociadas con la condición simpódica. Algunas veces en fetos simpódicos las dos arterias umbilicales permanecen casi por completo separadas. En otros casos solamente se pierde la porción distal de uno de los miembros del par umbilical. La llamada fusión de las arterias umbilicales para formar un solo vaso medial no puede, por esta causa, considerarse como la causa de la condición simpódica. El autor da una lista de los factores que entran en la formación de las arterias umbilicales variantes. El factor que produce la predisposición a la formación de arterias umbilicales impares que aparecen como ramas aórticas directas es la persistencia de las raíces umbilicales ventrales. Las arterias umbilicales impares que se originan en la aorta en un nivel alto y pasan directamente al ombligo, han sido interpretadas por muchos como arterias onfalomesentéricas persistentes. Estos vasos representan más probablemente arterias umbilicales primarias fusionadas. Las arterias umbilicales impares laterales que se originan en el tronco común iliaco-hipogástrico se han desarrollado normalmente. La ausencia de una arteria umbilical en el lado opuesto se debe a la atrofia de su porción distal después del establecimiento de su raíz dorsal o a la ausencia de la raíz umbilical primaria.

## THE ORIGIN AND OCCURRENCE OF THE SINGLE UMBILICAL ARTERY IN NORMAL AND ABNORMAL HUMAN FETUSES

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NINE TEXT FIGURES

### INTRODUCTION

The single umbilical arteries described in the literature can be divided into two groups. Group 1 includes all which arise from the abdominal aorta, whether as direct branches or as caudal continuations of the vessel itself; group 2, those which take origin from either the right or left common iliac-hypogastric trunk. Recently two cases of the occurrence of a single umbilical artery in full-term fetuses have come under my notice. These were representative of the two groups just mentioned. In one specimen (Dawson, '22), a sympodial monster, the single median umbilical artery arose directly from the aorta a short distance below the superior mesenteric artery (fig. 2). In the other, a normally formed fetus, only the left umbilical, appearing as a branch of the hypogastric artery, was present (fig. 1). These two cases, together with a rather extensive survey of the literature, form the basis of this paper.

A single umbilical artery is an almost constant feature of the fetal monstrosity sympodia, and the constancy with which it appears in this class of monster has led many to conclude that the single median umbilical artery is the primary anomaly and that the other concomitant anomalies are subsidiary to it. Johnston ('20) is probably the latest exponent of this theory. Ballantyne ('04), too, after a comprehensive survey of human monstrosities, pointed out that fetuses in which there is only one umbilical artery are generally malformed, and those in which the

single artery is mesial in position and arises directly from the aorta are nearly always malformed in one special way, namely, exhibit fusion of the lower limbs or sympodia. He, with many others, believed, furthermore, that the single median artery supplying the cord was vitelline in origin, being the persistent omphalomesenteric artery, and concluded like them that the arterial supply of the placenta was not an allantoic derivative. The latest writer to express this view of the vitelline origin of the single umbilical artery is Taglicht ('21).

Why there should be normally two umbilical arteries and only a single vein in the fetal cord is not clear. The venous supply of the placenta is primarily bilateral, but the median umbilical vein of fetal life is the persistent left umbilical of the embryo, the right having atrophied during early embryonic development. Furthermore, in the development of the vascular system of the higher vertebrates there is to be noted a general tendency for primitive bilateral vessels to be transformed secondarily into single, and sometimes median trunks. This is especially true if the vessels supply organs which, like the cord and placenta, are not bilateral.

In many cases it is difficult to determine the manner in which the transformation into a single vessel is effected. In some vessels it is evident that one member of the pair gained the ascendancy and its fellow atrophied consequently. In other cases the two vessels were probably completely fused to form a single trunk. Apparently, single umbilical arteries may be derived in either way, but the courses and relations of the single blood vessels are by no means identical. Moreover, the manner in which a single umbilical artery may be derived is also greatly complicated by several features of the normal development of these vessels. The primary umbilical arteries which rise ventrally from the abdominal aorta normally migrate from the cervical to the lumbar region. In the latter position (fig. 3) they acquire secondary connections with dorsolateral branches of the aorta, and following the establishment of these dorsal relations the primary or ventral connections with the aorta atrophy.

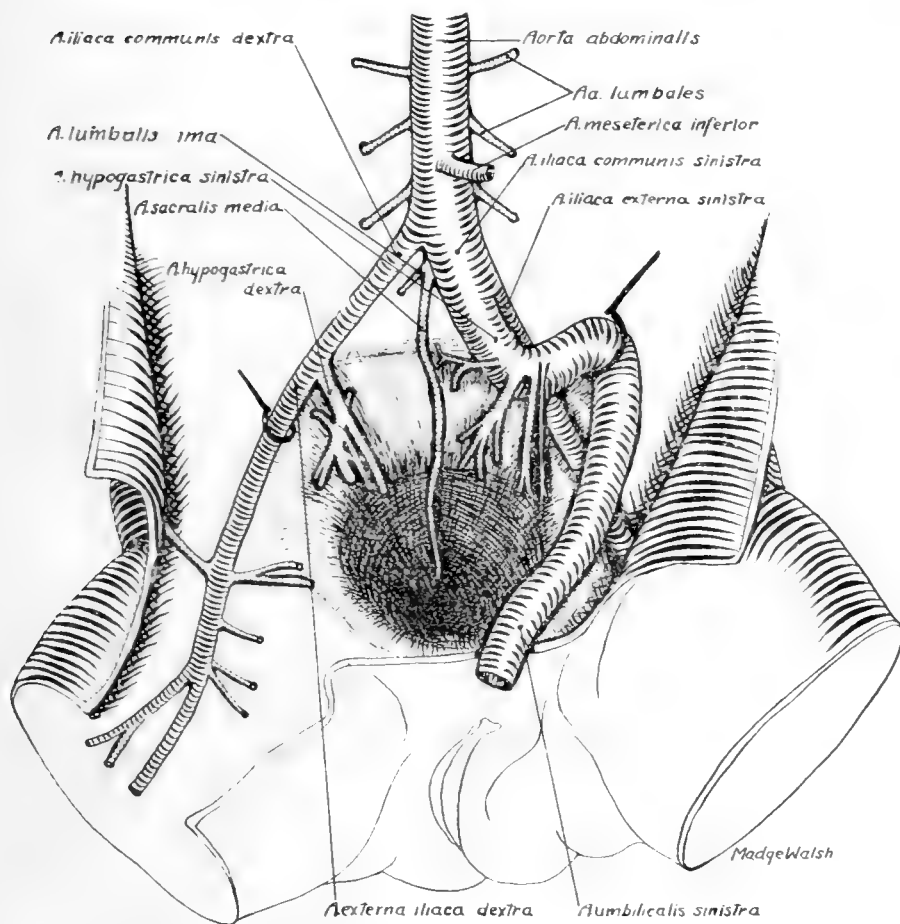


Fig. 1 Ventral view of the pelvis of a female fetus, showing the absence of the right umbilical artery. The relations of the right external iliac and the left umbilical arteries have been disturbed to display the smaller vessels distributed to the pelvic region. For complete description see text.

The substitution of dorsal roots for ventral roots may possibly explain why a single umbilical artery is not more commonly encountered in normal development. The dorsal segmenta pairs of vessels are never, in human embryos, converted second-

arily into single arteries, while the ventral vitelline series normally undergoes this transformation, and the umbilical or allantoic arteries are regarded as caudal members of the vitelline series. In cases, therefore, in which the dorsal connections are never established or if established fail to persist (and the primary ventral roots are accordingly maintained), it would be expected that the umbilical arteries would exhibit a tendency to duplicate the history of their homologous series, the paired vitelline arteries, and would be accordingly converted into single trunks. These single trunks would also necessarily arise directly from the abdominal aorta, and not from the common iliac-hypogastric trunk as they do if the normal plan of development is followed. Other cases, in which the single umbilical artery arises from either the right or left hypogastric, admit of another explanation. This will be discussed later.

From the preceding statements it will be evident that there is not, as yet, complete agreement regarding the origin and occurrence of the single umbilical artery in man, and in this regard I wish to present, in the succeeding pages, evidence in support of the following conclusions: 1) that a single umbilical artery, arising directly from the aorta, is not the primary anomaly in symphyodial monsters; 2) that the development of such a blood vessel is probably favored by the persistence of the original ventral umbilical roots; 3) that in order to satisfy the facts it is not necessary, except in rare cases (Ballantyne, '98), to interpret the single umbilical arteries belonging to group 1 as persistent omphalomesenteric arteries, and, 4) that single arteries of group 2 are the result either of the failure of one member of the umbilical pair to develop originally or of the secondary atrophy of the distal as well as the proximal portion of a primary artery on either the right or left side following the establishment of its dorsal connections. The latter of the alternative explanations seems the more probable one, although little direct evidence can be produced to substantiate it.

I wish to acknowledge my indebtedness to Dr. H. D. Senior for many helpful criticisms and suggestions. He is, however, in no way responsible for any of the conclusions drawn in this paper.

## A SINGLE UMBILICAL ARTERY IN A NORMALLY DEVELOPED FETUS

The subject of this description was a full-term female fetus used for dissection in a course in fetal anatomy. Interest in the condition of the umbilical arteries was aroused on observing a great difference in the size of the right and left common iliac arteries (fig. 1), the left being several times larger than the right. A careful search failed to reveal even a trace of a right umbilical artery. The right hypogastric and the right external iliac arteries, however, were normal in size and position. The right ovarian was abnormal and has already been described (Dawson and Reis, '22). The right superior vesical artery was also lacking. Four paired lumbar arteries were present, and on the right side an a. lumbalis ima was found.

The arrangement of the arteries from the left hypogastric conformed to what Piersol ('07) has designated fetal type II (p. 810, fig. 725), in which the three large vessels, the superior gluteal, the internal pudendal, and the inferior gluteal arise independently from the main (hypogastric) trunk (McMurrich, '20, p. 248, fig. 154). The single umbilical artery had practically the same caliber as the abdominal aorta.

## A SINGLE UMBILICAL ARTERY IN A SYMPODIAL MONSTER

The main features of the anatomy of this monster have already been briefly reported (Dawson, '22). The umbilical artery was single and approximately median. It arose from the abdominal aorta a short distance below the superior mesenteric artery (fig. 2). Below the point of origin of the umbilical artery the dorsal aorta was greatly diminished in size and gave off inferiorly only the lumbar arteries, second, third, and fourth right, and second and third left. It terminated in a small vessel suggestive of a middle sacral artery and did not have any further connection with the arterial supply of the pelvis and lower extremities.

The umbilical artery, on the other hand, was very large, equaling in size the vessel from which it arose. In the upper portion of the abdominal cavity it occupied a position slightly ventral

and lateral (left) to the dorsal aorta and was enclosed in a low peritoneal fold. More caudally, this fold was materially increased in height and in the pelvic region formed a sickle-shaped septum which partitioned the rudimentary pelvic cavity into right and left halves. The umbilical artery which was located in the free margin of this fold accordingly curved ventrally in the sacral region and then ascended toward the umbilicus on the anterior abdominal wall in the midventral line. The artery was displaced slightly to the left by the descending colon, which was distended with meconium, as the rectum and anus were absent. This displacement is somewhat exaggerated in figure 2 to show the courses and relations of the dorsal blood vessels.

Shortly after its origin from the aorta, the umbilical artery gave off an apparently normal inferior mesenteric artery, and in the sacral region a second and very prominent branch was found. It arose as a median dorsal trunk at the point of greatest convexity of the umbilical artery as it curved forward and upward to supply the umbilical cord. This dorsal branch was 1.5 cm. long and bifurcated in the shallow pelvis, giving rise to two large lateral vessels which, after giving off a few small arteries to supply the pelvis, continued into the 'fused' lower limbs.

#### A SCHEMATIC OUTLINE OF THE VARIOUS FACTORS INVOLVED IN THE FORMATION OF VARIANT UMBILICAL ARTERIES

The scheme, outlined below, is based primarily upon the normal ontogenetic plan of the umbilical arteries (Felix, '10; Hochstetter, '90; Senior, '19; Tandler, '03), but it is, in parts, supplemented by a study of two dissections I have made and by analyses of the many umbilical variants reported in the literature. The factors are as follows:

1. The failure of one member of the primary pair of umbilical arteries to develop.

2. Variations in the caudal migration of the umbilical arteries from the cervical to the lumbar region: *a*) arrested migration, the arteries arising from the aorta at a low thoracic or high lumbar level (fig. 4); *b*) complete or normal migration (fig. 3).



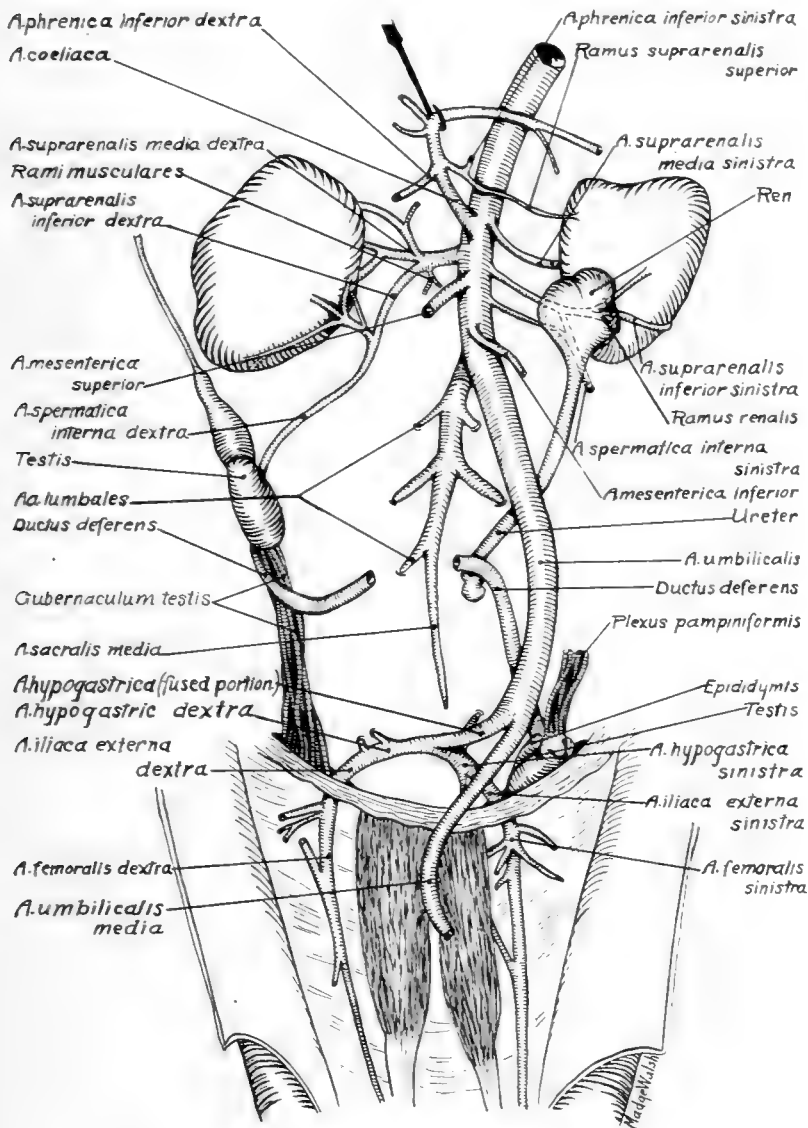


Fig. 2 Ventral view of the abdominal region of a symphytic monster, showing the anomalous arterial supply and the rudimentary urinogenital system. For description see text.

(There may also possibly be cases of normal migration followed by return, thus simulating arrested migration.)

3. Variations in the development of dorsal or secondary roots: *a*) failure of the dorsal roots to connect with the umbilical arteries (fig. 4), dependent to some extent on *2a*; *b*) normal dorsal root connections established with the primary paired umbilical arteries; *c*) unilateral development of the dorsal roots, in cases where both primary arteries are present or where only one member of the primary pair is present.

4. The varying fates of the primary (ventral) and the secondary (dorsal) roots in the condition *3b* or *3c*: *a*) loss of both primary roots and the persistence of the dorsal root or roots (normal); *b*) the persistence of both dorsal and ventral roots more or less completely; *c*) loss of the proximal portions (i.e., proximal to the point of origin of the primitive external iliac artery) of the dorsal roots and the persistence of the ventral roots; *d*) the loss of one dorsal and one ventral root; *e*) the persistence of two dorsal roots and one ventral root; *f*) the loss of one dorsal root and the persistence of two ventral roots.

5. The method of transformation of the paired umbilical arteries into a single vessel. (Although in many cases there is no direct evidence to indicate that the single umbilical arteries are derived from primitively paired vessels, still the fact that two dorsolateral connections were made has been accepted as indirect evidence of such a condition, since it hardly seems possible for a single median branch of the aorta to establish these connections.) *a*) By the loss of one umbilical artery distal to the point of origin of the external iliac artery. (It must be kept in mind, however, that the point of origin of the external iliac artery is not an absolutely fixed one, since this artery may also migrate either proximally or distally along the common iliac-hypogastric trunk.) *b*) By the fusion of the two umbilical arteries, influenced possibly by conditions represented in *2a*, *3a*, and *4b*, *4c*, or *4f* (fig. 5).

6. The varying fates of the lumbar and sacral portions of the abdominal aorta. The lumbar portion, *a*) persists in condition *3a* and *4a*, but, *b*) usually undergoes more or less atrophy caudal

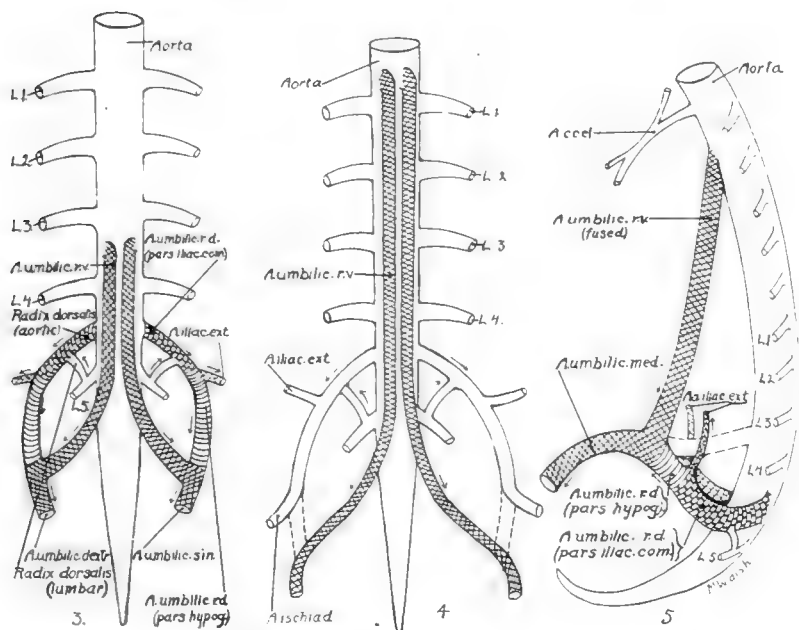


Fig. 3 Diagrammatic representation, ventral view, of the normal development of the arteries of the lower limbs and umbilical cord. Both dorsal and ventral roots of the umbilical arteries are indicated. The several portions of the arterial system of special interest in this paper are distinguished by hatching and stipple, and this method is adhered to uniformly in all subsequent figures (4 to 9). *r.v.*, radix ventralis; *r.d.*, radix dorsalis. The other abbreviations do not need explanation. Arrows indicate the direction of blood flow.

Fig. 4 Diagrammatic representation, ventral view, of the developing abdominal aorta, illustrating a condition in which the ventral umbilical arteries have failed to complete caudal migration and to establish secondary connections (dorsal roots) with the aorta.

Fig. 5 Diagram showing abnormal development of the abdominal aorta as seen from the left side. The ventral umbilical arteries have failed to migrate caudally and have fused to form a single median vessel. They have also established dorsal root connections with the aorta. The normal position of the ventral umbilical roots is shown by broken lines.

to the point of origin of the ventral umbilical roots in a condition represented by 2a and 5b or 4c. The sacral portion is, c) often missing, especially in symphyliad monsters but, d) may persist.

7. The possibility of a single umbilical artery or any other large single trunk, which arises from the aorta at the lower lumbar level to become terminal in position. The normal middle sacral is rarely terminal.

8. The possibility in cases where the original ventral roots are fused and the dorsal roots atrophy proximally, a) for the distal or persistent (hypogastric) portions of the dorsal roots to be transformed (apparently by fusion) into a single trunk which would accordingly take its origin from the dorsal aspect of the single umbilical artery. This fusion, however, b) may be incomplete or, c) may not occur at all.

#### REVIEW OF LITERATURE AND DISCUSSION

The review of the literature has been postponed until this time and the somewhat hypothetical explanation of single umbilical arteries interpolated between it and the description of the two dissections in order that the two cases described and the several cases reviewed might be utilized as cumulative evidence in support of the conclusions noted above. The discussion, it is hoped, will also be facilitated by having before us in a schematic form a list of the various factors which may possibly be effective in the formation of variant umbilical arteries. These factors will be referred to by the number and letter under which they are listed. No attempt will be made to review the literature completely. Only cases which are believed to have a direct bearing on the points under consideration have been selected.

A single median umbilical artery in direct continuity with the abdominal aorta has long been regarded as a constant feature of symphodia and its importance has been, I believe, to a large extent over-emphasized. Johnston ('20) and many earlier writers regarded the single vessel as the primary anomaly. They believed that it was not the persistent member of a pair, but was formed by the 'fusion' of the two umbilical arteries, and that it therefore acted both mechanically, by forming an obstruction to caudal development, and physiologically, by resulting in a deficiency in the vascular supply to the posterior end of the body.

(The word 'fusion' has been rather loosely used in the literature with little or no indication as to whether 'failure to develop separately' or 'secondary union following separate development' was meant. Unless it is indicated otherwise, as above, by means of quotation marks, the latter meaning is implied when the term is used here.)

If Johnston's suggestion, that the formation of a single median umbilical artery is the primary anomaly in symphodia and is directly responsible for the defective development of the caudal portion of the body, is correct, it would follow that in all cases of symphodia that there must always be but one artery and it must also be median in position. In reviewing the literature, however, it is obvious that the single umbilical artery in symphodial monsters is not always formed by 'fusion,' but is in some cases without question a persistent member of a pair of vessels. As early as 1889 Schwing demonstrated a case of symphus dipus in which "*Der Nabelstrang enthält nur eine Nabelarterie welche aus der linken A. iliaca entspringt*" (p. 485). This is apparently an ordinary case of unilateral persistence, with a secondary (dorsal) root, of one member of the umbilical pair. Later Odisio ('92) figured and described the abdominal arterial supply of a case of sirenomelus, in which it is evident that the single vessel supplying the cord is a persistent left umbilical artery (fig. 7). The arterial arrangement in this monster, however, is not so simple as in the case reported by Schwing, since the original ventral roots of both umbilical arteries have persisted. Duckworth ('07) has also described a case of low-grade symphus dipus (specimen E) with a single umbilical artery. There is good reason, too, for regarding this single vessel as a persistent left artery which takes its origin from the abdominal aorta by means of the primary or ventral root (fig. 9). A more complete consideration of these cases will be given later.

More recently, Langer ('21) described a case of symphodia in which the left umbilical artery was absent and the right appeared as a branch of the right common iliac-hypogastric trunk.

Besides the four cases of symphodia mentioned above which exhibit unilateral persistence of an umbilical artery, there is a

case of symphus dipus described by Gladstone ('06) in which the double arterial supply to the placenta was maintained practically complete. The umbilical artery arose as a single trunk from the abdominal aorta a short distance below the coeliac axis, but soon divided to form a large left and a small right umbilical artery. Neither of these umbilical vessels, however, had any connection with the common iliac-hypogastric trunk.

Single umbilical arteries have also been found associated with other monstrosities than symphodia. Hermann (1822) described two cases of congenital umbilical hernia in which there was a single umbilical artery arising from the aorta. Claudius ('59) reported a case of acephalus in which the umbilical artery appeared as a single branch of the aorta. Strassmann ('95) demonstrated a case of omphalocoele in which a single umbilical artery, arising from the left hypogastric, was present. Weil ('97) described a similar fetal defect with the right umbilical artery absent and the left appearing as a branch of the hypogastric. Enryrs-Roberts and Paterson ('06) described a case of ectopia viscerum in which the right umbilical artery appeared as a caudal continuation of the abdominal aorta. The left artery was not present.

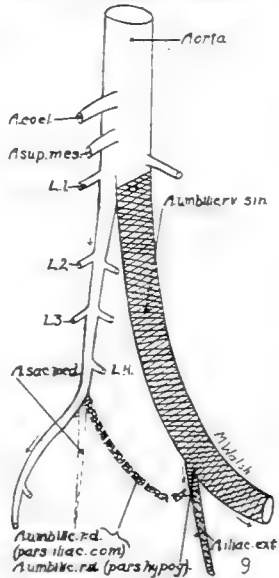
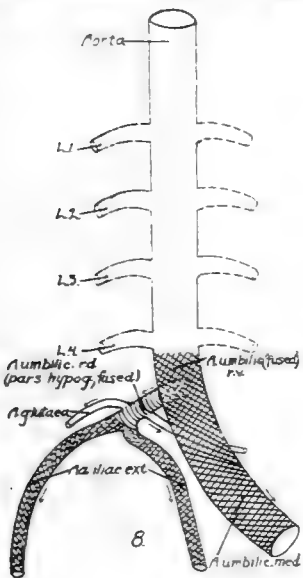
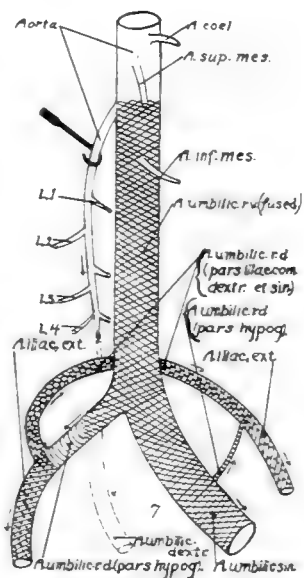
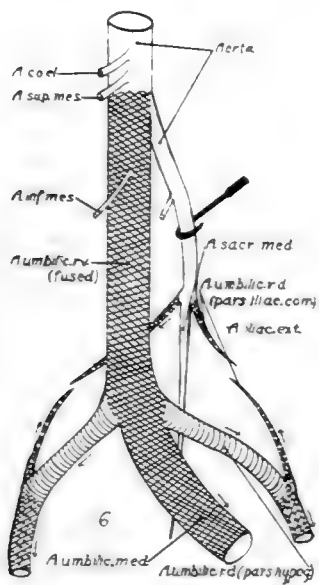
The cases reviewed above furnish, I believe, sufficient evidence to allow us to discount to a considerable extent the emphasis placed by many of the very frequent association of a single and median umbilical artery with the monstrosity symphodia. The formation of a single median artery by the 'fusion' of the original pair can scarcely be regarded as the primary anomaly in symphodia, since occasionally in this type of monster both umbilical arteries may remain almost completely separate, and more fre-

Fig. 6 Diagram suggesting an interpretation of the abdominal arterial system described by Duckworth ('07) for specimen F. (The labeling in this and all succeeding figures is the author's.)

Fig. 7 Diagram suggesting an interpretation of the anomalous abdominal arterial system figured by Odisio ('92).

Fig. 8 Diagram based on Johnston's ('20) description and figure of the arterial supply of a symphodial monster.

Fig. 9 Diagram suggesting an explanation of the anomalous arterial supply figured by Duckworth ('07) for specimen E.



quently only one member of the pair may persist. Furthermore, other types of fetal defects, as omphalocoele, in which the development of the caudal portion of the body is affected, often exhibit similar variations in the arterial supply of the placenta.

While a median umbilical artery is not invariably associated with sympodia, still it is the most common type of variant single umbilical artery found in this class of monster. The development of the median artery appears to be dependent on the persistence of the original ventral roots, whereby the umbilical arteries maintain the same relation to the abdominal aorta as do the vitelline arteries, and undergo the same changes, i.e., are transformed into single vessels. The umbilical arteries are regarded as caudal precocious members of the vitelline series.

The most simple type of median umbilical artery is represented by a combination of the factors 2*a*, 3*a*, and 5*b*, and is the one most commonly encountered in sympodial monsters (Taglicht, '21, et al.). The single artery arises from the abdominal aorta at the lower thoracic or upper lumbar level and passes almost directly to the umbilical cord, having no connections with the common iliac-hypogastric trunk (fig. 4). In these cases the primitive umbilical vessels did not complete the normal caudal migration, no secondary dorsal root connections were established, and the paired vessels were transformed into a single vessel in the same manner as the other paired members of the vitelline series. It is possible, however, that in these cases only one member of the primary pair developed originally, but there is indirect evidence, as already noted, that in many cases two umbilical arteries must have been present at an earlier stage of development. Furthermore, in the case described by Gladstone ('06) the degree of fusion is limited to the short portion adjacent to the aorta. Moorhead ('05) also reported a case (sympus apus) in which the umbilical artery arose from the aorta as a single trunk and entered the cord as a single trunk, but in the intermediate portion it became double for a short distance.

A single umbilical artery having the course and relations of the type under discussion has been interpreted by many (Taglicht, '21, et al.) as a persistent omphalomesenteric artery. In rare



cases (Ballantyne, '98) it seems barely possible that the allantoic circulation may be replaced by the vitelline circulation, but, unless there is sufficient evidence of modification in the placenta to justify such an interpretation, there appears to be no valid ground for advocating it. There is certainly nothing in the ontogenetic history to preclude the interpretation I have offered.

The relations of the single umbilical arteries, in the previous cases where no secondary dorsal connections were made with the aorta, are relatively simple. On the other hand, in cases where the secondary roots were established, followed by either abnormal loss or persistence of either the primary or secondary roots, the relations of the single artery become more complex and it is often difficult to recognize homologies. An additional complication is introduced by the manner of development of the common and external iliac and hypogastric arteries in relation to the dorsal umbilical root. The second case reported in this paper is of this more obscure type, and, in conjunction with my attempt to interpret this anomaly, I wish to discuss rather fully four somewhat similar cases which represent instructive transitional stages in the transformation of a primarily bilaterally symmetrical arterial system into a single median one.

Duckworth ('07) reported a case of *sympus dipus* ? specimen F, in which the abdominal aorta, below the superior mesenteric artery, divided into a large median ventral and a much smaller dorsal vessel (fig. 6). The ventral vessel appeared at first sight to be the caudal continuation of the abdominal aorta. The dorsal branch, however, was found to be the true aorta and it retained its original dorsal position, continuing caudally upon the vertebral column to end in a middle sacral artery, after giving off distinct branches to the right and left sides of the pelvis. The large ventral trunk arched forwards over the uterine portion of the cloaca and passed downward in the middle line over the rudiment of the bladder. Immediately above the symphysis pubis it gave off symmetrical branches to each side of the pelvis and then bent upwards to enter the umbilical cord as a single vessel. Each of the symmetrical right and left branches, after giving off a femoral branch at the appropriate point, was finally traceable

to the back of the pelvic cavity and came to an end in the close vicinity of the lateral terminal branches of the reduced dorsal aorta.

The condition of the abdominal arterial system in this case is represented by a combination of the factors 2*a*, 3*b*, 4*b*, 5*b*, 6*d*, and 8*c*. The large ventral vessel was produced by the fusion of the primary umbilical arteries which did not complete their caudal migration and retained their original ventral roots. The secondary or dorsal roots were also established, but they underwent considerable atrophy proximal to the point of origin of the external iliac arteries. They were represented in this portion (normally the common iliac portion) by the lateral branches of the dorsal aorta and the dorsolateral branches from the symmetrical right and left branches of the median ventral umbilical artery. These symmetrical branches are homologous with the hypogastric portions of the dorsal umbilical roots. The flow of blood in the latter vessels consequently must be in a direction opposite to that which it takes in the normal arteries. The lumbar portion of the dorsal aorta is also very much reduced in size, due to the diversion of the blood stream brought about by the retention by the umbilical arteries of their primary high origin.

A case similar in many respects to that described by Duckworth ('07) was figured by Odisio ('92). A large ventral artery arose from the aorta below the superior mesenteric artery and continued caudally for a considerable distance as a single vessel (fig. 7). In the region of the pelvis, however, it bifurcated into a small right and a larger left division. The right artery terminated in two relatively large branches; one went back into the pelvis, the other, the external iliac, supplied the right leg. The larger left artery continued practically undiminished in size to the umbilicus, and gave off along its course a small pelvic branch, which in turn gave off the left external iliac artery and then passed dorsally and medially into the pelvis to become continuous with a similar artery from the opposite side. A reduced dorsal aorta was present and it terminated shortly after giving off the lumbar arteries.

A combination of the factors 2*a*, 4*b*, 5*a*, and *b*, 6*b* and *c*, and 8*d* would produce a result quite similar to this case of Odisio's. The original ventral roots persisted, but failed to migrate caudally the normal distance, and fused for the greater portion of their course, being separate only in the pelvic region. Distal to its union with the right secondary root the right umbilical artery, however, had completely atrophied. The left persisted and became the single vessel of the umbilical cord. Both dorsal umbilical roots were retained, but their early connections with the dorsal aorta were lost, as the latter vessel terminated a short distance cranial to the level from which its dorsal branches originally arose. Although the portion of the abdominal aorta from which the secondary roots arose disappeared, the proximal ends of the roots have remained in continuity. Apparently most of the blood supplied to the left side of the pelvis and left leg must have followed a circuitous route through the vessel of the right side, which is made up of the unfused portion of the right primary umbilical root and the right secondary or dorsal root, reaching the left leg by way of the common iliac portion of the left dorsal root. It is obvious that the small left hypogastric element, of itself, could not supply the larger vessel going to the leg (fig. 7). With the exception of the mistake as to the large ventral artery's being the omphalomesenteric, both Duckworthe and Odisio offered much the same explanations as given above.

In the second case of single umbilical artery described in this paper (figs. 2 and 5) the arterial supply of the abdomen did not differ greatly from the two cases already discussed, and represented a condition such as a combination of factors 2*a*, 3*b*, 4*c*, 5*b*, 6*b*, and 8*b* would produce. The original ventral roots failed to migrate the normal distance caudally, and the primitive umbilical arteries were fused throughout their entire course. Secondary dorsal connections with the aorta were established, but they were subsequently lost proximal to the origin of the external iliac arteries. The portions lost would normally form the common iliac trunks (fig. 5). The distal persisting or hypogastric portions of the dorsal roots were incompletely fused, so that the blood supply of the rudimentary pelvis and 'fused' limbs arose

as a single vessel from the dorsal surface of the umbilical trunk, and later divided into a right and a left branch (fig. 2). From points near the bifurcation of the single trunk small arteries left the hypogastric portions of the right and left branches and passed to the viscera and walls of the shallow pelvis. The main branches continued as external iliac and femoral arteries to the 'fused' lower appendages.

A somewhat different type of single umbilical artery was described by Johnston ('20). The median umbilical artery in this case did not appear as a branch, but as the caudal continuation of the abdominal aorta (fig. 8). "The common iliac arteries were therefore indistinguishable, but the external iliacs arose by a common trunk from the dorsal aspect of the single median artery and almost at once separated, being normal in the remainder of their course so far as could be traced. Their common trunk gave off a few small branches to the rudimentary pelvis and a single gluteal on each side" (p. 211). The arrangement of the abdominal arterial system in this case could be produced by a combination of factors 2*b*, 3*b*, 4*c*, 5*b*, 6*c*, 7 and 8*a*. In contrast with the three cases previously considered, the fused primary umbilical roots have migrated the normal distance caudally. The proximal portions of the secondary roots have atrophied, and along with the fusion of the primary umbilical arteries a fusion of the distal or hypogastric portions of the dorsal roots has also occurred. Because of the loss of the proximal connections of the dorsal roots and the normal lumbar position of the fused primary roots, the lower lumbar and sacral portions of the abdominal aorta have completely disappeared and the single umbilical trunk has assumed secondarily a terminal position.

Another single umbilical artery, having much the same relations as the one described by Johnston ('20), was reported in the same year by Lange, the major difference being that the distal persistent or hypogastric portions of the dorsal umbilical roots did not fuse to form a single trunk. The so-called abdominal aorta accordingly underwent a tripartite division into two lateral vessels supplying the pelvis and legs and a large median artery which turned up and entered the umbilical cord.

The division occurred low in the pelvis some distance caudal to the bifurcation of the inferior vena cava. The proximal ends of the lateral branches of the abdominal aorta, according to my interpretation, are each composed of the hypogastric portion of the dorsal umbilical root and the primitive external iliac artery which grew out from the secondary root. The rôle of the axial or ischiadic artery in the development of the femoral or distal part of these vessels is not taken into account.

In all cases of single median umbilical arteries reviewed there is therefore evidence that the primitive ventral roots have persisted, and it seems probable that this persistence has favored the fusion of the originally paired vessels. Occasionally, however, even in cases where both primary roots are retained one member of the umbilical pair may undergo atrophy distally (Odisio, '92). In a case described by Duckworth ('07), specimen E. to which reference has already been made (p. 331), only the left umbilical artery was present. It arose from the dorsal aorta immediately below the first lumbar arteries and passed caudally to the umbilical cord giving rise to a small gonadic artery and a larger vessel which went to the left leg. The interpretation is suggested in figure 9. The primary umbilical root on the left side persisted and migrated caudally only to a level between the first and second lumbar arteries. The left dorsal umbilical root disappeared proximal to the origin of the external iliac artery and the distal persisting or hypogastric portion linked the external iliac with the left umbilical artery. The history of the right side is more obscure. It is entirely possible that only the left umbilical artery made its appearance originally, otherwise we would have to surmise an atrophy of the primary or ventral umbilical root and a persistence of the secondary or dorsal root—a complete reversal of what occurred on the left side. In the absence of any direct evidence, I prefer the latter interpretation.

In other cases where apparently both secondary umbilical roots were established and the primary roots subsequently atrophied, single arteries are produced by the disappearance distally of one member of the umbilical pair (fig. 3), and the persistent artery appears as a branch of the common iliac-

hypogastric trunk. A somewhat similar condition could also be produced by the failure originally of one member of the umbilical pair to develop. Lateral umbilical arteries of this type have been described in both normal and monstrous fetuses. Schwing ('89) and Langer ('21) reported cases in sympodial monsters. Weil ('97) found this condition associated with congenital omphalocele. Duckworth ('07) described a persistent left umbilical artery in an abnormal fetus (specimen A). De Archangelo ('01) and Mouchotte ('00) found unilateral persistence of the umbilical arteries in normal fetuses, and the case described in this paper (fig. 1) was found in a normally formed female fetus.

In my specimen (fig. 1) a right lumbar ima was present, and on the same side the umbilical artery was absent. Normally the lumbar ima is more often absent than present (Levy, '02), and, according to Senior ('19), the chief share in the early formation of the dorsal root of the umbilical artery is taken by a vessel which appears as a branch from the fifth lumbar segmental artery. The early atrophy of the distal portion of the right umbilical artery, which presumably occurred in this case, and the consequent diversion of the placental stream to the artery of the opposite side may possibly explain why this fifth lumbar segmental artery was not obliterated during the formation of the right common iliac trunk.

#### SUMMARY AND CONCLUSIONS

The single umbilical arteries described in the literature can be divided into two groups; group 1, including all which arise directly from the abdominal aorta; group 2, those which take origin from the common iliac-hypogastric trunk.

Two cases representative respectively of these two groups are described in this paper. The one belonging in group 1 was found in a sympodial monster, the other was present in a normally formed female fetus.

Single median umbilical arteries arising from the abdominal aorta are associated only with sympodia. Occasionally, however, in sympodia the two umbilical arteries remain almost completely separate. In other cases of sympodia the distal portion of one

member of the umbilical pair is lost and only one artery is accordingly present in the cord. These exceptions indicate, therefore, that the so-called fusion of the umbilical arteries to form a single median vessel cannot be directly responsible for the monstrous condition.

A list of the several factors which may be concerned in the formation of variant umbilical arteries is given.

The predisposing factor for the formation of single median umbilical arteries, which appear as direct aortic branches, is the persistence of the primary or ventral umbilical roots.

Single umbilical arteries, arising from the abdominal aorta at a high level and passing directly to the umbilicus without any connection with the arterial supply of the lower limbs, have been interpreted by many as persistent omphalomesenteric arteries. These vessels, however, more probably represent fused primary umbilical arteries which did not migrate caudally or become secondarily connected with the aorta at a lower level by means of the so-called dorsal roots. Consequently, such vessels can have no connections with the iliac and hypogastric arteries, which must accordingly develop independently.

Lateral single umbilical arteries which arise from the common iliac-hypogastric trunk have followed the normal plan of development. The absence of an umbilical artery on the opposite side is due either to the atrophy of its distal portion following the establishment of its dorsal connections with the aorta or to the failure of the primary umbilical root to appear originally. In the latter case the dorsolateral vessel leaving the aorta between the fourth and fifth lumbar segments must form the blood supply of half of the pelvis and one leg without any association with the umbilical arterial supply.

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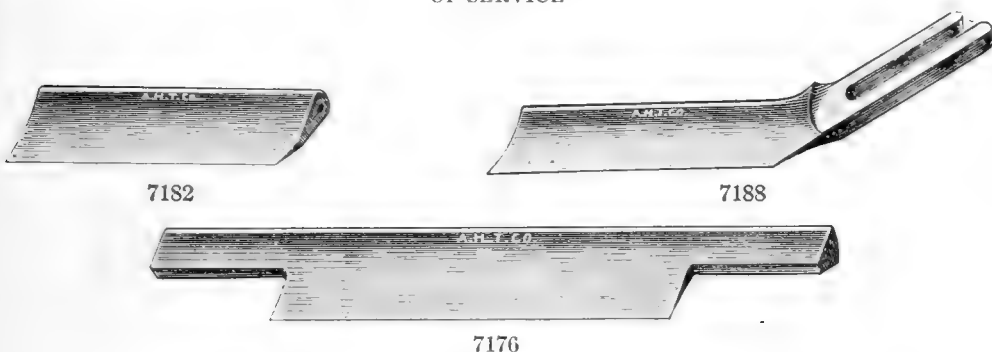


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219

# MICROTOME KNIVES

THE TEST  
IN OUR STOCK FOR IMMEDIATE SHIPMENT  
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Our microtome knives are specially made for us under our direction in Philadelphia, by workmen of long experience. They are forged, ground and tempered with exceeding care, and while some slight variation is to be expected as to relative hardness and thickness in different knives, they are all within the limits for satisfactory section cutting and sharpening.

Our method of grinding these knives provides that the cutting edge and the edge of the back lie in exactly the same plane, a very important feature in the longer knives when used obliquely as in celloidin sectioning, and also in sharpening.

Most difficulties in satisfactory section cutting are due to the condition of the knife edge because of unskilled attempts at sharpening. A microtome knife is usually considered to be unfit for use if it will not easily clip a human hair held by one end, and for many kinds of work even this is not a satisfactory guarantee of keenness. The cutting edge of a knife can also be easily examined under a low power on the microscope stage, and when it has been properly sharpened the edge will be seen to be smooth and even.

**7182. Microtome Knives, Plain** for use with Freezing and Rotary Microtomes. With threaded hole in which to screw Handle for sharpening.

Length of blade, mm.....	90	125	165
Length of cutting edge, mm.....	82	120	158
Each, in case.....	5.85	8.80	11.40
Code Word.....	Keoqm	Keorc	Keozu

**7188. Microtome Knife, Shankd.** The original form of knife designed for use with all Sliding "Sledge" Microtomes. In this type of knife, the chief difficulty has arisen in the past through the fact that the lower surface of the shank has not always been found to lie in the same plane as both the cutting and the back edge. This feature is carefully observed in the manufacture of these knives.

Length of blade, mm.....	90	125	165
Length of cutting edge, mm.....	82	120	160
Each, in case.....	10.10	10.90	14.80
Code Word.....	Keuhz	Keulr	Keuok

**7176. Microtome Knife, Minot Precision**, designed specially for use with the Minot Precision Microtome. The handles, by which the knife is clamped in the microtome, are extensions of the back proper, of which they are a part and have the same cross-section; 315 mm. long with a cutting edge of 190 mm.

In case.....	\$25.00
Code Word.....	Keuvc

Prices subject to change without notice

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The publication of reference cards bearing authors' abstracts began with articles appearing in The Wistar Institute journals of June, 1917.

In July, 1920, the preparation of reference cards, without abstracts, was begun for all the earlier articles which have appeared in the

Journal of Morphology from vol. 1, 1887, to June 1, 1917;  
The Journal of Comparative Neurology, from vol. 1, 1891, to June 1, 1917;  
The American Journal of Anatomy from vol. 1, 1901, to June 1, 1917;  
The Anatomical Record from vol. 1, 1906, to June 1, 1917;  
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This series of reference cards, forming a complete author's index to the above journals, will be finished as rapidly as possible and will be sent to subscribers as they are issued.

From volume 1 of each journal to the commencement of the Bibliographic Service cards, with the authors' abstracts (June 1, 1917), there will be issued—

For the Journal of Morphology . . . . .	Cards for 443 titles
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Please send to the address below, as they are issued, the reference cards for the following journals. (Cross out those not desired.)

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Additional cards as follows:

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Devoted primarily to the interests of anatomy as taught in the Medical Schools of the United States and to the development of scientific anatomy in these schools. Publishes researches only.

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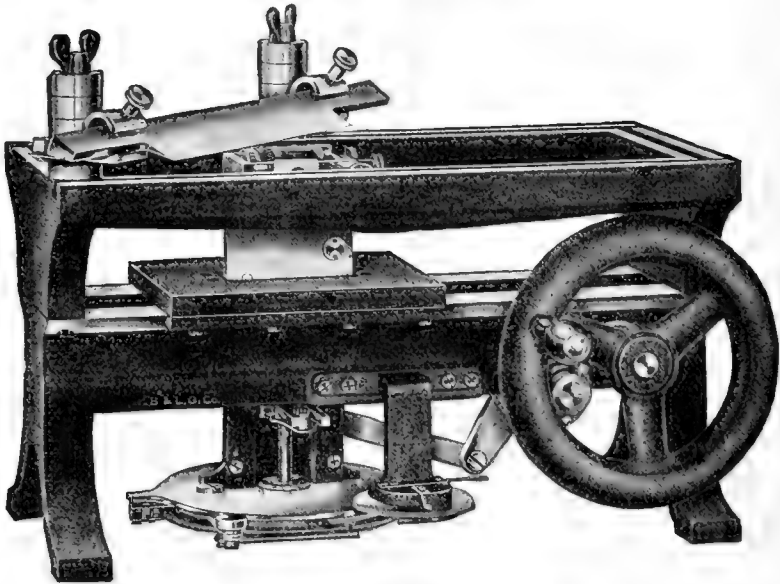
Every paper accepted for publication in one of the above periodicals is accompanied by the author's abstract. The abstract is printed on one side and the complete bibliographic reference to the paper as it will eventually appear is printed on the reverse side of a standard library catalogue card. These cards are issued in advance of the journal containing the paper.

Cards of the Bibliographic Service covering all papers published in the six journals for the year ending June 1, 1923, sent postpaid for \$5.00. Cards for previous years can also be supplied.

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The gear construction insures a steady stroke and even contact with the feeding mechanism, eliminating, to this extent, the possibility of personal error. The feeding mechanism operates automatically and provides for cutting sections 1 micron and more up to 25 microns in steps of single microns. Recent improvements are greatly increased rigidity of frame construction and the exceptionally long range of feed.

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PROCEEDINGS OF THE AMERICAN SOCIETY OF  
ZOOLOGISTS

The American Society of Zoölogists held its Twentieth Annual Meeting at the Massachusetts Institute of Technology in conjunction with Section F of the American Association and in association with other biological societies, particularly the Botanical Society of America, Ecological Society of America and the American Society of Naturalists, December 27, 28 and 29, 1922.

The officers for the year were:

- President: HARRIS H. WILDER.
- Vice-President: BENNET M. ALLEN.
- Secretary: W. C. ALLEE.
- Treasurer: DAVID H. TENNENT.
- Chairman, Genetics Section: HERBERT S. JENNINGS.
- Secretary, Genetics Section: LEON J. COLE.
- Local Committee: R. P. BIGELOW, Chairman, G. H. PARKER, H. W. RAND AND H. V. NEAL.
- Executive Committee: M. M. METCALF, GEORGE LEFEVRE, C. M. CHILD, GILMAN A. DREW AND CHARLES A. KOFOID.

*Representatives of the Society in the Division of Biology and Agriculture of the  
National Research Council*

	<i>Term expires</i>
H. S. JENNINGS .....	1925
WILLIAM PATTEN .....	1924
F. R. LILLIE .....	1923

*Membership of the Council of the A.A.A.S.*

CHARLES ZELENY  
HENRY E. CRAMPTON

EDITORIAL BOARD OF THE JOURNAL OF MORPHOLOGY

*Managing Editor* (Term expires 1926).....C. E. McCLUNG

*Associate Editors*

To serve until 1923.....	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">E. G. CONKLIN</div> <div style="display: inline-block; vertical-align: middle;">M. F. GUYER</div> <div style="display: inline-block; vertical-align: middle;">W. M. WHEELER</div> </div>
To serve until 1924.....	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">C. A. KOFOID</div> <div style="display: inline-block; vertical-align: middle;">F. R. LILLIE</div> <div style="display: inline-block; vertical-align: middle;">J. T. PATTERSON</div> </div>
To serve until 1925.....	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">L. L. WOODRUFF</div> <div style="display: inline-block; vertical-align: middle;">G. A. DREW</div> <div style="display: inline-block; vertical-align: middle;">H. V. NEAL</div> </div>

The Nominating Committee (Art. III, Sec. 6), composed of H. V. Neal, H. H. Newman, and Helen Dean King, reported the following nominations:

*President:* M. F. GUYER.

*Vice-President:* R. A. BUDINGTON.

*Member Executive Committee:* H. H. WILDER.

*Member National Research Council:* E. G. CONKLIN.

*Associate Editors of the Journal of Morphology:* H. V. WILSON, D. H. TENNENT, and CASWELL GRAVE.

*Membership in the Council, A. A. A. S.:* CHARLES ZELENY and H. E. CRAMPTON.

Nominations from the floor were called for, but none being presented these officers were unanimously elected.

The resignation of D. H. Tennent as Treasurer of the Society was referred to the Executive Committee with instructions to attempt to secure clerical assistance such that Dr. Tennent might continue in office or otherwise to appoint his successor.

The Executive Committee reported the nomination of the retiring vice-president, B. M. Allen and S. O. Mast to serve on the committee on cooperation with the National Research Council. They were duly elected. This committee is now composed of the following members: F. R. Lillie (chairman) and Wm. E. Castle, who serves one more year; C. A. Kofoid and D. H. Tennent, who serve two more years; A. A. Treadwell and A. A. Schaeffer, who serve three more years, and the newly elected members who will serve four years.

The following zoölogists, having been duly nominated, were recommended for membership in the Society by the Executive Committee as having shown sufficient evidence to warrant the belief that they would continue active in zoölogical research



- AGERSBERG, HELMAR PARELI VON WOLD KJERSCHOW, S.B., S.M. (Washington), A.M. (Columbia), Instructor in Zoölogy, *University of Nebraska, Lincoln, Nebraska.*
- BEHRE, ELINOR HELENE, A.B. (Radeliffe), Ph.D. (Chicago), Assistant Professor of Zoölogy, Louisiana State University, *Box 70, University Station, Baton Rouge, La.*
- BUCHANAN, JAMES WILLIAM, B.S. (Ohio), Ph.D. (Chicago), Instructor in Biology, Yale University, *778 Orange St., New Haven, Conn.*
- CHIDESTER, FLOYD EARLE, Ph.B. (Syracuse), Ph.D. (Clark), Associate Professor of Zoölogy, *West Virginia University, Morgantown, W. Va.*
- COLLINS, HENRY HOMER, A.B. (Rochester Normal), S.B., A.M., Ph.D. (California), Assistant Professor of Biology, University of Pittsburgh, *144 De Soto St., Pittsburgh, Pa.*
- DUNN, EMMETT REID, A.B., A.M. (Haverford), Ph.D. (Harvard), Assistant Professor of Zoölogy, *Smith College, Northampton, Mass.*
- FAUST, ERNEST CARROLL, Ph.D. (Illinois), Associate in Parasitology, Union Medical College, Peking, China. Address 1923-1924, *School of Hygiene and Public Health, Johns Hopkins University.*
- HIGGINS, GEORGE MARSH, S.B. (Knox), A.M., Ph.D. (Illinois), Assistant Professor of Biology, Knox College, *65 W. North St., Galesburg, Ill.*
- HYMAN, ORREN WILLIAMS, A.B., A.M. (North Carolina), Ph.D. (Princeton), Professor of Histology and Embryology, College of Medicine, University of Tennessee, *College of Medicine, Memphis, Tenn.*
- NICHOLAS, JOHN SPANGLER, S.B., S.M. (Gettysburg), Ph.D. (Yale), Assistant Professor of Anatomy, *University of Pittsburgh, Pittsburgh, Penna.*
- NOBLE, GLADWYN KINGSLEY, A.B. (Harvard), Ph.D. (Columbia), Associate Curator in charge of Herpetology, American Museum of Natural History, *77th St. and Central Park West, New York City.*
- PLOUGH, HAROLD HENRY, A.B. (Amherst), Ph.D. (Columbia), Associate Professor of Zoölogy, *Amherst College, Amherst, Mass.*
- RILEY, CHARLES F. C., A.B. (Doane), S.B. (Michigan), A.M. (Nebraska), Assistant Professor of Zoölogy, *University of Manitoba, Winnipeg, Manitoba.*
- SPEIDEL, CARL CASKEY, Ph.B. (LaFayette), Ph.D. (Princeton), Associate Professor of Anatomy, *University of Virginia, University, Va.*
- STRONG, LEONELL C., B.S. (Allegheny), Ph.D. (Columbia), Associate Professor of Biology, St. Stephens College, *Annandale-on-Hudson, New York.*
- WALTON, ARTHUR CALVIN, B.A., M.A. (Northwestern), Professor of Zoölogy (on leave), North-Western College, *906 S. First St., Champaign, Illinois.*
- WEINSTEIN, ALEXANDER, B.S., Ph.D. (Columbia), Johnston Scholar, Johns Hopkins University, *Biological Laboratory, Johns Hopkins University, Baltimore, Md.*
- WHEELER, GEORGE CARLOS, B.A. (Rice Institute), M.Sc., D.Sc. (Harvard), Instructor in Entomology, *Department of Zoölogy, Syracuse University, Syracuse, New York.*
- WILLIER, BENJAMIN HARRISON, B.S. (Wooster), Ph.D. (Chicago), Instructor in Zoölogy, *Department of Zoölogy, University of Chicago, Ill.*

The following former members were reinstated:

- COKER, R(OBERT) E(RVIN), B.S., M.S. (North Carolina), Ph.D. (Hopkins), Professor of Zoölogy, University of North Carolina, *Chapel Hill, N. C.*  
 GRIGGS, LELAND, A.B., Ph.D. (Dartmouth), Professor of Biology, Dartmouth College, *Hanover, N. H.*

## UNION OF BIOLOGICAL SOCIETIES

The proposed federation of biological societies was approved and, on nomination by the Executive Committee, F. R. Lillie and W. C. Allee were appointed to represent the Society on the council of the new organization. In order to avoid confusion this federation will be known as the Union of American Biological Societies.

### TREASURER'S REPORT

The report of the Treasurer for the year 1922 was examined and approved by the auditing committee, W. H. Longley and A. A. Schaeffer, and was accepted and ordered placed on file by the Society. The report follows:

#### *Report of Treasurer of the American Society of Zoölogists for the Year 1922*

Balance on hand last report, Dec. 26, 1921.....	\$ 908.09
Additional receipts by Treasurer Allee between date of report and date of remittance to new Treasurer.....	102.85
	<hr/> \$1,010.94
Additional expenditures by Treasurer Allee.....	6.20
	<hr/> 1,004.74
Balance forwarded to D. H. Tennent, Jan. 15, 1922.....	2,327.53
Receipts Jan. 15 to Dec. 19, 1922.....	<hr/> 3,332.27
Total cash handled during 1922.....	2,488.15
	<hr/>
Balance on hand, Dec. 19, 1922.....	\$ 844.12
The funds of the Society are deposited with the Bryn Mawr Trust Co., Bryn Mawr, Pa.	
Savings Account.....	\$ 711.65
Checking Account.....	132.47
	<hr/>
Total.....	\$ 844.12

## ANALYSIS OF REPORT OF TREASURER

*Receipts*

Received from W. C. Allee, Jan. 15, 1922.....		\$1,004.74	
Back dues received .			
1 at \$ 2.00.....	\$	2.00	
3 at 5.00.....		15.00	
11 at 7.00.....		77.00	
2 at 11.50.....		23.00	
		<hr/>	
Total.....	\$	117.00	\$ 117.00
Dues current year received:			
71 at \$ 5.00.....	\$	355.00	
212 at 7.00.....		1,484.00	
28 at 11.50.....		322.00	
		<hr/>	
Total.....	\$2,161.00		2,161.00
Dues of Irregular Amount.....		7.00	
Refund for Overpaid Subscriptions.....		6.00	
Dues for 1923 and 1924 paid in advance.....		21.50	
Interest Credited.....		15.03	
		<hr/>	
Total Income 1922.....		\$2,327.53	2,327.53
		<hr/>	
Total cash handled.....			\$3,332.27
Total expenditures.....			2,488.15
			<hr/>
Balance.....			\$ 844.12

*Expenditures*

Paid for Current Expenses of Secretary:			
Expenses Secretary at Toronto.....	\$	39.05	
“ “ Typewriting.....		9.30	
“ “ Jan.-March.....		23.66	
“ “ Printing (Wistar).....		64.61	
		<hr/>	
Total for Secretary.....	\$	136.62	\$ 136.62
Paid S. I. Kornhauser, as voted by Society in aid of work on dyes.....			25.00
Paid L. J. Cole, share of A.S.Z. for printing in connection with Genetics.....			22.75
Paid A. O. Weese, Ecological Society of America, charge against appropriation voted by A.S.Z. ....			12.70
Expenses Treasurer, Postage.....			10.60
Refunded for overpaid dues.....			4.50

## Paid Wistar Institute, Subscriptions:

Feb. 25....\$ 210.50	\$1,748.50	
Mar. 8.... 291.00	May 8.... 118.00	
" 10.... 296.50	June 15.... 122.50	
" 15.... 262.50	" 27.... 37.00	
" 20.... 196.00	Aug. 7.... 45.50	
" 25.... 216.00	Sept. 25.... 44.00	
April 5.... 160.00	Oct. 14.... 67.50	
" 17.... 116.00	Nov. 9.... 37.00	
	Dec. 18.... 55.00	
	<hr/>	
\$1,748.50	\$2,275.00	\$2,275.00
Exchange on foreign checks.....		.98
Total Expenditures.....		\$2,488.15
The actual income of the Society during the year 1922 has been:		
From 16 payments of back dues at .50.....	\$ 8.00	
From 311 payments of current dues at .50.....	155.50	
Interest on deposits to June 30.....	14.59	
Interest accrued but not yet credited.....	14.66	
	<hr/>	
Total.....	\$ 192.75	
The expenses of the Society have been:		
Secretary's office.....	\$ 136.62	
Grant to S. I. Kornhauser.....	25.00	
L. J. Cole, Genetics.....	22.75	
Grant, Ecological Society.....	12.70	
Treasurer's office.....	10.60	
	<hr/>	
Total.....	\$ 207.67	
To this amount should be added \$33.50 paid to The Wistar Institute by order of the Executive Committee in cancellation of the bad debts of two members of the Society who were dropped for non-payment of dues.....		
	\$ 33.50	
Actual Expenditures.....	241.17	
Actual Income.....	192.31	
	<hr/>	
Excess of expenditures above income.....	\$ 48.86	

This amount (loss) will be increased when total expenditures of Secretary and of Treasurer are paid.

Three ex-members (in addition to the two mentioned above) dropped for non-payment of dues, each in arrears for the years 1919-20-21, owe the Society a total of \$63.00; three members in arrears for 1921 and 1922 owe a total of \$38.00; 28 members in arrears for 1922 only, owe a total of \$188.50; this making a grand total of \$289.50.

D. H. TENNANT, Treasurer.

December 19, 1922.

The Secretary reported that President Wilder had appointed H. S. Pratt to represent the Society in the matter of the Spencer Fullerton Baird Memorial.

E. F. Adolph, also appointed by President Wilder, represented the Society at the Conference on World Metric Standardization which was held at Pittsburgh in September under the auspices of the American Chemical Society, submitted the following report:

It appears that organized opposition to the popular use of the metric system in this country has arisen. It was not the purpose of this Conference to oppose this, but to act in encouraging and crystallizing the influence of scientists in this matter. There was no discussion upon the use of legal means in attaining the spread of the metric system. All those present were in favor of a very gradual adoption of the system, through the agency of educational institutions chiefly. Commercial institutions can not be influenced in any wholesale fashion, but the system of double marking which is already widespread should be encouraged. Four resolutions embodying these sentiments were adopted.

The report of the secretary of the Conference, Professor W. V. Bingham of Carnegie Institute of Technology, has been published in *Science*, vol. 56, page 362, September 29, 1922.

#### REPORT OF COMMITTEE ON BIOLOGICAL STAINS.

To the Members of the Society of American Zoologists:

Your representative on the Committee on Biological Stains wishes to report the following progress:

Sixteen members of the society agreed to carry on tests to compare American made dyes for microscopical purposes with European products. Seven basic dyes and five acid dyes were selected for these tests, it being the aim to include only those dyes most essential for general laboratory work. Mr. R. T. Will of Rochester and your representative then mailed out 203 samples to the following collaborators:

Ezra Allen,	Ursinus College	Bordeaux Red	7
-		Acid Fuchsin	6
L. B. Arey,	Northwestern Univ.	Haematoxyline	3
		Eosin	7
Gary N. Calkins,	Columbia University	Methyl Green	7
F. W. Carpenter,	Trinity College	Methylene Blue	16
Ulric Dahlgren,	Princeton Univ.	Haematoxylin	3
		Orange G	6
H. S. Davis,	Univ. of Florida	Bordeaux Red	6
Robert Hance,	North Dakota Agri. College	Haematoxylin	3
		Eosin	7
R. W. Hegner,	Johns Hopkins Univ.	Methyl Green	6
		Eosin	7

Davenport Hooker,	Univ. of Pittsburgh	Methylene Blue	8
		Eosin	5
S. I. Kornhauser,	University of Louisville	Haematoxylin	3
		Orange G	7
		Safranin	7
C. E. McClung,	Univ. of Pennsylvania	Safranin	8
		Haematoxylin	2
		Orange G	7
G. H. Parker	Harvard University	Mythelene Blue	26
F. P. Reagen,	Univ. of California	Haematoxyline	3
		Eosin	7
W. R. B. Robertson,	Univ. of Kansas	Haematoxylin	3
		Orange G	6
George L. Streeter,	Johns Hopkins Univ.	Carmine	4
		Cochineal	2
		Carmine Acid	2
H. B. Ward,	Univ. of Illinois	Eosin	10
		Congo Red	7
		Orange G	2

Each sample of the test stains was given a number according to its manufacture or source: thus no. 250 stood for Grüber, no. 223 Coleman and Bell, no. 123 National Aniline and Chemical Company, etc. No investigator except your representative was informed as to the source of the dyes he tested. In each case when obtainable Grüber's dyes were included and many of the collaborators included in their tests as checks Grüber stains which they had in their own laboratories, and with which they were familiar.

Thirteen collaborators have sent in their final reports to Chairman H. J. Conn and myself. These thirteen reports cover all the kinds of stains sent out. The reports in general are very satisfactory and show that in many instances American dyes are superior to those formerly used. Thus no. 250 is often far down in the list arranged according to desirability.

American C. P. Haematoxylin has proved entirely satisfactory. Domestic Methylene blue for blood stains, for tissues, and for intra vitam work of the most delicate sort has proved its worth. The results on carmine and its allied stains were good. In regard to safranin and methyl green, opinions differ, there being one favorable and one unfavorable report for each. Of the acid dyes good American products of all types tested have been found. In regard to Orange G, the American dye is often far more concentrated than that formerly used and tends to overstain in alcoholic solutions.

Inasmuch as the work of the Committee on Biological Stains operated under the National Research Council and obtained its funds through the agency of the Research Council and since the Council did not wish to pass judgment on commercial products, the committee together with the majority of collaborators has formed a new organization independent of Research Council, a commission to certify biological stains. Dr. H. T. Conn has been the prime mover of this arrangement.

It is planned to draw up specifications for the various dyes, to test samples submitted and to sell the producer or bottler of the dye labels certifying the contents.

The coöperation of the members of our Society in this work has been splendid and their detailed results are available to any who may chance to use them.

Respectfully submitted,

(Signed) S. I. KORNHAUSER.

The report was discussed at length. Dissatisfaction was expressed over the proposal to sell certification certificates to producers, and the attention of the Society was called to the possibility of legal complications growing out of the publication of decisions of the committee which might tend to force firms out of business. The whole question was referred to the Executive Committee with power.

### *Resolutions*

The committee on resolutions consisting of H. S. Pratt, Alice Boring and C. E. McClung presented their report which was adopted by a standing vote and ordered spread on the records of the Society.

The SOCIETY OF ZOOLOGISTS desires to record in the following minutes its recognition of the services to science and to humanity of the members who have died during the past year:

#### JAMES VISCOUNT BRYCE

In the selection of honorary members the American Society of Zoölogists has been most conservative but in electing Lord Bryce as the first of this group, it set a worthy standard. It is not necessary here to make record of the well known achievements of this great man, but it is well to remind ourselves of the great value of the scientific method of the study of human relationships, so well exemplified by him in his masterly historical studies. His example should be an inspiration to all who would make the methods and principles of science generally effective in social relations.

The American Society of Zoölogists desires to make formal record of the profound respect in which the character and achievements of Lord Bryce are held by its members, and to express the great sense of loss which is felt in his passing.

#### ALFRED GOLDSBOROUGH MAYOR

1868-1922

Dr. Mayor was one of the most brilliant and versatile American men of science. A keen, analytical and finely trained mind, great industry, and a high courage which led him to undertake any matter he was interested in without regard to the obstacles or dangers involved, enabled him to produce scientific work of enduring value, and

also, as Director of the Department of Marine Biology of the Carnegie Institution of Washington, to put many other investigators in the way to do the same. A refined and artistic temperament, a knowledge of books and a wide experience of men and affairs, an appreciation of what is fine and beautiful in life and the world, and a fund of good nature, made him a friend and companion almost beyond compare. He was a unique personality, and his death has left a gap in our world of science which cannot be filled; we shall not see his like again.

#### CAROLINE BURLING THOMPSON

1869-1921

In spite of much ill health Dr. Thompson achieved the all too unusual result of being both a productive scholar and an inspiring teacher. Her work on the Termites has placed the origin of the castes of these forms on a new and sound basis. This work was pursued in the midst of hours of teaching with a steadfastness of purpose which was, along with her ever stimulating personality, one of the characteristics by which she will long remain in the minds of her students and scientific colleagues; to them her example will not cease to be a stimulus and an incentive.

#### ALICE ROBERTSON

It was not only through her many investigations on the Bryozoa, by which she is widely known, but through the embodiment in her of the spirit of the scholar and the scientist, that Dr. Robertson made her influence felt. Though research was her life, hers was a many-sided mind, bringing to the study of political events and of literature the same keen interest and understanding which was manifest in her special field; and to all this was added the human kindliness, that ever-ready Scotch wit, and her joy of life, that drew to her such a host of friends.

#### *Changes in the Constitution and By-Laws*

The Constitution of the Society was amended to allow the election of associate members from the workers in zoölogy who do not satisfy the research requirements for full membership. The By-Laws were changed to provide, first, that the annual dues from such associate members would be the same as for full members and, second, that such associate members might become members of sections of the Society and are entitled to the journal privileges of members. The By-Laws as amended do not extend the program privileges of the Society to associate members unless they are introduced by members. The Constitution and By-Laws as amended are published in another section of the Proceedings.



## REPORT OF THE COMMITTEE ON PUBLICATION, AMERICAN SOCIETY OF ZOOLOGISTS, 1922

By vote of this society in 1919 the three incoming associate editors of the *Journal of Morphology* constitute a Consulting Committee, whose function it is to assist in maintaining a spirit of helpful cooperation between the Society of Zoölogists and The Wistar Institute, and to consider problems relating to the publication of research in zoölogy.

Your committee reports that as a result of the increase in numbers of papers presented for publication in the Wistar journals since the Great War, affairs have reached a crisis like that which forced the Society in 1916 to increase its annual dues to \$6.50. The fact is that the funds available for the publication of researches in zoölogy have long been inadequate to meet the demand. The attempt of the management of the Wistar to meet this emergency in 1921 resulted in a deficit of \$11,406.65 or more than one quarter of the total income of the Institute. With the income at present available it is possible to publish less than half of the papers presented for publication. This presents a serious problem since the prompt publication of the results of research is of vital importance to every active zoölogist. Some solution of the problem presented in this accumulation of unpublished material is urgently needed. Matters cannot be permitted to continue as at present.

It may not be fairly said that this is a problem for the Wistar to solve and not a problem of our Society. The assumption of the burden of publication by the Wistar has meant much in the way of opportunities for the publication of our researches, but it does not mean the assumption of the entire burden of financial responsibility. The Wistar is under no obligations whatever to subsidize zoölogical research.

During the fourteen years since the Wistar undertook the publication of the Biological journals the publication of the researches of American zoölogists has been continuous and stable to a degree previously unknown. The Wistar has consistently met the essential requirements for 'prompt publication in adequate form with the widest possible distribution to those interested in the results of zoölogical research.' In this period the subscription lists of the Wistar journals have risen from 1,410 to 5,286 in 1920, and the cost has increased from \$12,568.34 to \$46,005.25. The number of pages published annually has trebled. A marked improvement in the condition of manuscripts received for publication with consequent decrease in cost of corrections has followed the educational campaign carried on by the Wistar. The admirable bibliographic service has been added to the publication of the abstracts of the papers presented at the annual meetings. The great service performed by the Wistar Institute and its efficiency as a publication office are too well known to require emphasis at this time. But not even the Wistar can be expected to continue to subsidize the publications of the zoölogists of this country to the amount of \$11,000 annually.

In 1913 a Committee was appointed by this Society to raise funds for the publication of zoölogical research and for the support of the Wistar journals. But the outbreak of the Great War prevented the committee from accomplishing its purpose. The financial emergency was temporarily met in 1916 by the increase of the Society dues to \$6.50. For this sum—that is, for a sum less than the actual cost of publi-

cation—each member of this Society has received journals whose subscription price amounts to over fifty dollars. What this has meant is better appreciated when we remember that the many members of the American Chemical Society pay annual dues of \$15.00 and receive only three journals including the Chemical Abstracts, and the members of the British Chemical Society for an annual fee of sixteen dollars receive only a single journal.

The publication problem which confronts us is fundamentally a financial problem. since the congestion of unpublished matter can be relieved only by funds adequate for its publication. The system of fixed annual dues of this society does not permit the necessary increase in the amount of material published annually. The Wistar has been subsidizing the publication of research to the extent of several thousand dollars annually. Since we may not expect the Wistar to finance increasing deficits indefinitely, we must make up our minds as to what we intend to do.

Various suggestions have been made looking to the solution of our problem:—

1. It has been suggested that instead of receiving a group of journals each member be allowed the privilege of purchasing any of the Wistar publications for 50 per cent. of the established price.

2. Some urge further increase in the annual dues as a temporary expedient as in 1916. The Director of the Wistar estimates that in order to continue the present arrangement whereby members receive a group of journals and to permit an increase in the amount of material published annually it would be necessary to increase the annual dues to \$16.00.

3. Others recommend the appointment of a Committee to raise endowment for the publication of research.

4. Some believe that the financial problem could be solved by requiring authors or the institutions with which they are connected to share the cost of publication. In this way increase in revenue would accompany increase in material published.

5. Several suggestions looking towards greater economy in publication, but they do not appear to meet the fundamental difficulty. Some recommend a stricter limitation in the length of papers, or a more rigorous editorial policy, the limitation of publication privileges to members of this Society, the elimination of plates except when the author pays for them, and the substitution of the bibliographic cards in the place of the abstracts of papers, etc. Such suggestions are obviously not mutually exclusive and might all be tried. While commendable they do not appear adequate to meet the present emergency.

6. Finally there is Dr. Crozier's plan of radical changes in the methods of publication involving

- a. The discontinuance of the grouping of papers in the numbers of a journal.
- b. The immediate publication of each accepted paper as a "separate."
- c. The discontinuance of the author's privilege of purchasing reprints at cost.
- d. The sale of individual papers at prices as near the cost of publication as possible.

Whatever the advantages—or disadvantages—of this plan, the Committee believes it to be illusory as a device for meeting the financial problem of increased amount of publication. Indeed it is the conviction of the committee that hasty action of any sort should not be taken. The problem must be carefully considered in all its aspects and decision reached only after it is certain that we shall not leap

from the frying pan to the fire. The need for immediate consideration is, however, so great that the Committee recommends that the President appoint a committee on publication with power to take whatever action seems necessary. This committee should confer with a similar committee of the Anatomists and of the Union of Biological Societies in case such a Union is formed.

Signed

GILMAN A. DREW,  
L. L. WOODRUFF,  
H. V. NEAL.

After some discussion, which included a statement from Dr. Greenman concerning the point of view of The Wistar Institute, the following resolution was adopted addressed to the Editorial Board of the *Journal of Morphology* and to The Wistar Institute:

*Resolved:* That the American Society of Zoölogists favors a policy of condensation of papers, including those already in hand, until the present congestion in printing of research results be relieved and that preference in publication be given with reference to condensation.

On motion of the Executive Committee, the Society appointed a Publication Committee consisting of C. E. McClung, Chairman, F. R. Lillie, Caswell Grave, H. V. Wilson and D. H. Tennent and gave them power to make all temporary arrangements and negotiations necessary to continue publication up to the point of final commitment of the Society to new policies.

The thanks of the Society were voted to the local committee, R. P. Bigelow, G. H. Parker, H. V. Neal and H. W. Rand, in appreciation of their work in arranging for the meeting.

### *Minutes of Genetics Sections*

Sessions for the reading of papers were held Wednesday morning and Thursday morning and afternoon. In the absence of the chairman, Dr. H. S. Jennings, at the first session, Dr. C. B. Davenport was elected to preside. In addition to the reading of scheduled papers, the only business transacted was the appointment of a committee to nominate officers for the ensuing year. Dr. Jennings presided on Thursday. At a short business session during the afternoon the following officers were nominated and elected:

*Chairman*—E. M. EAST.

*Secretary*—D. F. JONES.

*Representatives of the Zoölogists*—J. H. GEROULD.

*Representative on the Board of Control of Botanical Abstracts*—SEWALL WRIGHT.

The secretary presented three recommendations from the Executive Committee, which were adopted:

1. Papers placed on the program shall be limited to a maximum of 15 minutes, except by special vote of the Executive Committee.
2. The final date for the reception of titles for the program shall be determined by the secretary and may be independent of the time set by the other sections of the parent societies. Titles, to be accepted, must be accompanied by abstracts of not to exceed 250 words in length.
3. In the absence of the author, papers on the program shall be read by title only, except by special vote of the members of the Sections present at the meeting.

The necessity of having small dues to meet the ordinary expenses of the Sections was presented by the secretary and it was

*Moved:* That annual dues of fifty cents per member be assessed for this purpose, the manner of its collection to be determined by the secretary in consulting with the officers of the parent Societies. Carried.

The question was raised as to whether the traveling expenses of the secretary in attending the annual meeting should be paid from the funds thus raised and the chairman ruled that such was the intent of the motion, to take effect the coming year.

The average attendance at the meetings was about 120.

LEON J. COLE,

Secretary of Genetics Sections.

#### PROGRAM

##### WEDNESDAY MORNING SESSION, DECEMBER 27

10:00 A.M. This session was held in three sections:

- I. Papers on Parasitology and on Cytology and Histology; twelve papers were read in full and eleven by title. Attendance 75-100.
- II. Miscellaneous papers; four papers were presented in full. Attendance 25-50.
- III. Joint Genetics Sections of Botanists and Zoölogists; twelve papers on animal genetics were presented in full and two were read by title.

##### WEDNESDAY AFTERNOON SESSION

2:00 P.M. Papers on Embryology and Comparative Anatomy; thirteen papers were presented in full and three by title. Attendance 100 or more.

WEDNESDAY EVENING

9:00 P.M. The Biological Smoker was held in the Walker Memorial Building. About 500 present.

THURSDAY MORNING SESSION, DECEMBER 28

9:30 A.M. This session was held in two sections:

- I. Remaining papers on Comparative Anatomy and those on Entomology and those on General Physiology (in part); ten papers were read in full and eleven were read by title.
- II. Joint Genetics Sections: thirteen papers were read and three were presented by title. Attendance 100 or more.

THURSDAY AFTERNOON SESSION

- 2:00 P.M. I. General Physiology concluded with eleven papers read in full and twelve by title. Attendance about 100.
- II. Joint Genetics Sections; the program was concluded with 12 papers read in full and three by title.

THURSDAY EVENING

7:00 P.M. Zoölogists' Dinner followed by the address of C. A. Kofoed, retiring President of the Society and retiring Vice-President of Section F on "The Life Cycle of the Protozoa." Attendance 140.

FRIDAY MORNING SESSION, DECEMBER 29

- 9:15 A.M. Business.
- 10:20 A.M. Joint meeting with Ecological Society of America. Ten papers presented in full and five by title.

FRIDAY AFTERNOON SESSION

- 2:00 P.M. Joint symposium with the American Society of Naturalists and the Ecological Society of America. The entire program of nine papers was presented.

At the sessions for the presentation of papers, 106 papers were presented in full and 56 were read by title. There were eight exhibits making a total of 164 separate titles on the program.

LIST OF TITLES

The following titles, contributed for the program, have been grouped and arranged in accordance with rules accepted by the Society except in the Genetics program where cooperation with the Botanical Society have caused some slight modification.

The papers marked with an asterisk were read by title only.

The titles have been numbered consecutively and the corresponding abstracts has the same number given the title. On account of complications growing in the main, out of intersociety relations, certain abstracts were received too late to be included among those originally printed. They are given here in order to present

a complete report of the proceedings of the meeting but are grouped together as "late abstracts" in order to avoid the expense of breaking paging in matter already composed.

## A. PARASITOLOGY

- \* 1. Development of a myxosporidian, *Myxosoma catostomi* nov. spec. R. Kudo, University of Illinois.
- 2. *Spirochæta eurygyrata*. M. J. Hogue, North Carolina College for Women.
- 3. A new liver fluke from the monkey. Horace W. Stunkard, New York University.
- 4. Observations on an acquired immunity to a metazoan parasite. Leslie B. Arey, Northwestern University Medical School.
- \* 5. Variations in *Euglenamorphæ hegneri*, n.g., n.sp., from the intestine of tadpoles. D. H. Wenrich, University of Pennsylvania.
- \* 6. *Diphyllobothrium parvum*. Thomas Byrd Magath, Mayo Clinic.
- \* 7. Notes on Acanthocephala from Japan. H. J. Van Cleave, University of Illinois.
- \* 8. The relations between the food of frog and toad tadpoles and their intestinal protozoa. R. W. Hegner, Johns Hopkins University.
- 9. The use of the pure culture and the guinea pig in biological studies of parasitic nematodes. B. H. Ransom, U. S. Bureau of Animal Industry.
- \*10. Studies on *Necator suillus*, parasitic in pigs. James E. Ackert and Florence K. Payne, Kansas State Agricultural College.
- 11. The life-history and development of the mite, *Myobia musculi*, Schrank. Howard E. Enders, Purdue University.
- 12. The effect of carbon tetrachloride on intestinal protozoa. A preliminary note. M. J. Hogue and C. Van Winkle.
- 13. A new genus of trematode from the eastern painted turtle. Horace W. Stunkard, New York University.
- \*14. Cross-infection of muscoid flies with *Herpetomonas*. Elery R. Becker, Johns Hopkins University. (Introduced by W. H. Taliaferro.)
- \*15. Interactions between protoplasmic masses as a means of determining physiological variations in *Arcella*. B. D. Reynolds, Johns Hopkins University. (Introduced by R. W. Hegner.)
- \*16. Variations in *Taenia saginata*, the "beef" tapeworm of man. Franklin D. Barker, University of Nebraska.
- \*17. The chromosomes in *Ascaris lumbricoides* of man. Franklin D. Barker.

## B. CYTOLOGY AND HISTOLOGY

- 18. Cellular elements in the perivisceral fluid of the Echinodermata. James E. Kindred, Western Reserve University.
- 19. The application of the Bielchowsky-Paton method to the nervous system of the earthworm. (Lantern and microscopic demonstrations.) W. M. Smallwood, Syracuse University.
- \*20. Luteal cells in the gonads of the Phalarope (by title). Harry B. Yocum, University of Oregon.

21. The nature of the division of neuroblastic cells in the regenerating spinal cords of Amphibian larvae. Davenport Hooker, University of Pittsburgh School of Medicine.
22. Sources of nutriment during the metamorphosis of fresh-water mussels. Leslie B. Arey, Northwestern University Medical School.
23. The typical form of polyhedral cells in plant parenchyma and in human epithelium. (Lantern and models.) Frederic T. Lewis, Harvard Medical School (introduced by Herbert W. Rand).

## C. UNCLASSIFIED

24. Fiji-New Zealand expedition from the University of Iowa. (Lantern; 60 minutes.) C. C. Nutting, University of Iowa.
25. Observations of biological science in Russia. H. J. Muller, University of Texas.
26. The proper wording of titles of scientific articles. E. W. Gudger, American Museum of Natural History.
27. Variations of coat-color within a single subspecies of mice of the genus *Peromyscus*. (Lantern.) H. H. Collins, University of Pittsburgh. (Introduced by E. F. Adolph.)

## D. ENTOMOLOGY

- \*28. Afferent and efferent pathways in *Dendroides*. William A. Hilton, Cornell University.
29. Ant larvae. (Lantern.) W. M. Wheeler, Harvard University, and G. C. Wheeler, Syracuse University.
- \*30. Further observations on the digestive system of the periodical Cicada. Charles W. Hargitt and L. M. Hickernell, Syracuse University.

## E. EMBRYOLOGY

31. Periodicity in the production of sexual cells in marine animals. B. H. Grave, Wabash College.
32. Germ cell and germ gland development in male *Rana catesbeiana* tadpoles. (Lantern.) W. W. Swingle, Yale University.
33. The microdissection of the egg of *Cerebratulus*. George A. Baitsell, Yale University.
- \*34. The presence of copper in *Arbacia* eggs. Otto C. Glaser, Amherst College.
- \*35. Analysis and interpretation of lithium effects in echinid embryos. J. W. MacArthur, University of Toronto.
36. Cryptorchidism experimentally produced. Carl R. Moore, University of Chicago.
37. Iodine and anuran metamorphosis. (Lantern.) W. W. Swingle, Yale University.
38. Iodine and urodele metamorphosis. W. W. Swingle.
39. Experiments on metamorphosis. (Lantern.) Karl E. Mason, Yale University (Introduced by W. W. Swingle).

40. The vascularity of the enamel-organ in the developing molar of the albino rat. William H. F. Addison and J. L. Appleton, Jr., University of Pennsylvania. (Introduced by C. E. McClung.)
41. Experiments on limb posture in *Amblystoma punctatum*. J. S. Nicholas, University of Pittsburgh School of Medicine. (Introduced by R. G. Harrison.)
- 42.<sup>1</sup> The Entrance of the Spermatozoa into the Starfish Egg. Robert Chambers, Cornell University Medical College.
- \*43.<sup>1</sup> The Subdivisions of the Neural Folds in Man. G. W. Bartelmez, University of Chicago.

## F. COMPARATIVE ANATOMY

44. The origin and nature of the earliest neuromuscular connections in Elasmobranch embryos. H. V. Neal, Tufts College.
45. An undescribed type of sense organ found in the larva of *Botryllus*. Caswell Grave, Washington University, and Helen Woodbridge, University of Maine.
46. The morphogenesis of spines and spine-glands in the Siluridae. H. D. Reed, Cornell University.
47. On specific characters in *Teredo*. (Lantern.) Thurlow C. Nelson, Rutgers College.
48. Intramuscular sensory endings of the small intestine, with a consideration of their central connections and probable function. F. W. Carpenter, Trinity College.
- \*49. Glochidial teeth and the mechanics of attachment. Leslie B. Arey, Northwestern University Medical School.
50. The gall-bladder in the cat,—its development, its functional periodicity, and its anatomical variation as recorded in twenty-five hundred specimens. Edward A. Boyden, Harvard Medical School.
51. The anatomy of an abnormal double monster (Duroc) pig. (10 min.) George W. Hunter and George M. Higgins, Knox College.

## G. GENERAL PHYSIOLOGY

- \*52. The creeping of the larvae of the slug-moths (Cochliidiidae). W. J. Crozier, Rutgers College.
- \*53. Photoreceptors of *Lumbricus terrestris*. Walter N. Hess, DePauw University.
54. Hibernation in Orthoptera. I. Physiological changes during hibernation in certain Orthoptera. Joseph Hall Bodine, University of Pennsylvania.
55. Some reactions of *Alligator mississippiensis*. Albert M. Reese, West Virginia University.
- \*56. Quantitative observations on the digestive power in Amphibian larvae during metamorphosis. Albert Kuntz, St. Louis University School of Medicine.
- \*57. The effect of light on various marine invertebrates. A. G. Huntsman, Atlantic Biological Station.
- \*58. The axial gradients in *Corymorpha palma*. C. M. Child, University of Chicago.

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<sup>1</sup> See late abstracts.



- \*59. Oxygen consumption with respect to level, size, and regeneration and electrical polarity in *Corymorpha palma*. (By title.) L. H. Hyman, University of Chicago.
- \*60. The manner of copulation in triclad Planarians. R. A. Budington, Oberlin College.
- 61. The olfactory sense of the cabbage butterfly, *Pieris rapae* Linn. Dwight E. Minnich, University of Minnesota.
- \*62. The stimulating efficiency of intermittent light in the drone-fly, *Eristalis tenax*. William L. Dolley, Jr., Randolph-Macon College.
- 63. The chemical sense of *Palaemonetes vulgaris* (Say). Manton Copeland, Bowdoin College.
- \*64. Sterility of animals under changed conditions. (By title.) Carl Hartman, University of Texas.
- 65. The attachment of oyster larvae. (Lantern.) Thurlow C. Nelson, Rutgers College.
- 66. The circus movements of *Limulus*. William H. Cole, Lake Forest College.
- \*67. Effect of thyroid feeding on the color and form of the feathers of fowls. Benjamin Horning and Harry Beal Torrey, University of Oregon.
- \*68. The physiological response of *Paramecium* to thyroxin. Matthew C. Riddle and Harry Beal Torrey, University of Oregon.
- 69. Excretion and death amongamebas. A. A. Schaeffer, University of Tennessee.
- 70. The physiological action of excretory products. Edward F. Adolph, University of Pittsburgh.
- \*71. Eyes in *Volvox* and their function. S. O. Mast, Johns Hopkins University.
- 72. The growth of marine organisms on submerged metals. G. H. Parker, Harvard University.
- \*73. Relative effects on ciliary activity of anion and undissociated molecule of organic acids. J. M. D. Olmsted and J. W. MacArthur, University of Toronto.
- \*74. On the nervous organization of *Limax*. W. J. Crozier, Rutgers College.
- \*75. Concerning laws of locomotion in gasteropods. W. J. Crozier.
- \*76. Effect of thyroid feeding on the moulting of fowls. Benjamin Horning and Harry Beal Torrey, University of Oregon.
- 77. The reactions of larvae of *Vanessa antiopa* Linn. to sound. Dwight E. Minnich, University of Minnesota.
- \*78. The process of photic orientation in *Volvox*. S. O. Mast, Johns Hopkins University.
- \*79. On certain determining factors in regeneration. J. William Buchanan, Yale University. (Introduced by L. L. Woodruff.)
- \*80. Weight changes and oxygen consumption during long exposure to dilute anesthetics. J. William Buchanan.
- \*81. Amoeboid movement and coalescence of dissociated sponge cells. Paul S. Galtsoff, U. S. Bureau of Fisheries. (Introduced by W. C. Allee.)
- S2. Reversal of vertebrate heart beat. James Nelson Gowanloch, Wabash College. (Introduced by B. H. Grave.)
- S3. Observations on the ciliary action of *Scyphidia*. Ruth Jane Ball, University of Vermont. (Introduced by H. F. Perkins.)

- \*84. The effect of temperature upon the rate of retinal pigment migration in crustaceans. Rudolf Bennitt. (Introduced by G. H. Parker.)
- 85. The feeding reactions of the Ciliate, *Dileptus gigas*, with special reference to the function of the trichocysts. J. Paul Visscher, (Introduced by S. O. Mast,) Johns Hopkins University.
- \*86.<sup>1</sup> Reactions of Hydra to chloretone. William A. Kepner and D. L. Hopkins, University of Virginia.
- \*87.<sup>1</sup> Blood and nerve as controlling agents in the movements of melanophores. Leland C. Wyman. (Introduced by G. H. Parker.)

## H. ECOLOGY AND ZOOGEOGRAPHY

- \*88. Quantitative aspects of association and of seasonal succession in an artificial environment. W. J. Crozier and E. S. Harris, Rutgers College.
- 89. Some myriapods of South Bass Island, Ohio. Stephen R. Williams, Miami University.
- 90. American Opalinidae. (Charts.) Maynard M. Metcalf.
- 91. The alternative color-phases of fishes. W. H. Longley, Goucher College.
- \*92. Further observations on the hydrogen-ion concentration of Chesapeake Bay water. R. P. Cowles, Johns Hopkins University, and A. M. Schwitalla, St. Louis University.
- 93. The bibliography of fishes. E. W. Gudger, American Museum of Natural History.
- 94. The effect of environmental conditions on the rate of development. (Lantern.) A. O. Weese, James Millikin University.
- 95. Studies on animal aggregations: The temperature relation with isopods. W. C. Allee, University of Chicago.
- 96. The tadpoles of the frogs of Okefinokee Swamp, Georgia. A. H. Wright and A. A. Wright.
- 97. A morphological mechanism in some instances of physico-chemical adaptation. F. H. Pike, Columbia University.
- \*98.<sup>1</sup> Caddisfly larvae of swift and standing waters. (Lantern.) G. S. Dodds, West Virginia University, F. L. Hisaw, Kansas Agricultural College.
- 99.<sup>1</sup> An octo-flagellate parasitic in trout. (Lantern; 20 min.) Emmeline Moore, New York State Conservation Commission, E. S. A.
- \*100.<sup>1</sup> Recent migrations of southerly species of fish into northern waters. (Canadian Atlantic waters.) Edward E. Prince, Commissioner of Fisheries, Canada, A. S. Z.
- \*101.<sup>1</sup> The conversion of transverse stripes into longitudinal stripes in the coloration of some larval fishes. (Charts.) Edward E. Prince, Commissioner of Fisheries, Canada.

## I. GENETICS SECTIONS

- 102. The transmission of the polycladous character in *Sphaerocarpus Donnellii*. (Lantern; 15 min.) Charles E. Allen, University of Wisconsin.
- \*103. Color changes in maize pericarp and the nature of the gene. (Lantern, reflectoscope; 20 min.) William H. Eyster, University of Missouri.

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<sup>1</sup> See late abstracts.

- \*104. Inheritance of a primitive sporophyte in maize. (Lantern; 5 min.) William H. Eyster, University of Missouri.
- 105. Notes on heritable endosperm defects in maize. (8 min.) E. W. Lindstrom, Iowa State College.
- 106. Eight years selection for quality of oil in soy beans. (12 min.) L. J. Cole, E. W. Lindstrom, and C. M. Woodworth.
- 107. The relation between chromosome number and morphological characters in wheat hybrids. (8 min.) Karl Sax, Maine Agricultural Experiment Station.
- 108.<sup>1</sup> Induction of chromosomal mutants and their recognition in *Datura*. Albert F. Blakeslee and M. E. Farnham.
- 109.<sup>1</sup> Induction of gene and chromosome mutations in *Datura* by exposure to radium rays. (Lantern.) C. Stuart Gager and A. F. Blakeslee.
- 110.<sup>1</sup> Distribution of chromosomes in tetraploid daturas. J. Belling and A. F. Blakeslee.
- 111. Anatomical differences between the various chromosomal mutants of *Datura*. E. W. Sinnott and A. F. Blakeslee.
- 112.<sup>1</sup> Globe mutants to normal plants in *Datura* after pollination with counted pollen. J. T. Buchholz and A. F. Blakeslee.
- 113.<sup>1</sup> Species in the genus *Rubus* and *Crataegus*. Albert E. Longley.
- 114.<sup>1</sup> On a gigantic natural hybrid of the silverweed (*Potentilla anserina*). E. C. Jeffrey.
- 115.<sup>1</sup> On the origin of the boston fern. E. C. Jeffrey.
- 116.<sup>1</sup> Further evidence of linkage with crossing over in *Oenothera*. (15 min.) George H. Shull.
- \*117.<sup>1</sup> A preliminary report on the genetics of *Clarkia elegans*. (15 min.) (Lantern.) Leonas L. Burlingame.

## I'. GENETICS SECTIONS

- 118. Factors which determine otocephaly in guinea pigs. (Lantern; 15 min.) Sewall Wright, U. S. Department of Agriculture.
- 119. Persistent new color patterns in grouse locusts by mutation, and linkage, or homozygosis, with isolation. (Charts; 15 or 20 min.) Robert K. Nabours, Kansas State Agricultural College.
- \*120. The consequences of different degrees of interference, in the crossing-over of the hereditary genes. (15 min.) H. S. Jennings, Johns Hopkins University.
- 121. Coincidence of crossing-over in *Drosophila virilis*. (15 min.) Alexander Weinstein (introduced by H. S. Jennings), Johns Hopkins University.
- 122. Notes on the pigment cells in the eyes of *Drosophila* eye-color mutants. (15 min.) O. A. Johannsen, Cornell University.
- 123. Analysis of "contamination" in *Habrobracon*. (15 min.) P. W. Whiting, University of Iowa.
- 124. Facts indicating abnormal fertilization in *Habrobracon*. (15 min.) Anna R. Whiting and P. W. Whiting, University of Iowa.

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<sup>1</sup> See late abstracts.

- \*125. The birth rate among the graduates of Allegheny College. (By title), H. R. Hunt, University of Mississippi.
- 126. Sex-ratios in guinea-pigs. (Lantern; 10 min.) Heman L. Ibsen and Lucella Schaumburg.
- 127. An environmental factor causing variation in weight at birth of guinea-pigs. (Lantern; 10 min.) Heman L. Ibsen, Kansas State Agricultural College.
- 128. A brief description of abnormalities observed in the descendants of X-rayed mice. (10 min.) C. C. Little, University of Maine, and H. J. Bagg, Memorial Hospital, New York.
- 129. Inheritance of an eye-abnormality appearing in the descendants of X-rayed mice. (10 min.) C. C. Little, University of Maine, and H. J. Bagg, Memorial Hospital, New York.
- 130. The inheritance of a lethal headabnormality appearing among the descendants of X-rayed mice. (10 min.) C. C. Little, University of Maine, and H. J. Bagg, Memorial Hospital, New York.
- 131. A report of a histological study of the eyes and gonads of mice treated with a light dosage of X-rays. (5 min.) L. H. Snyder, M. Schneider and C. C. Little.
- 132. The production of non-disjunction by X-rays and the hereditary transmission through untreated females of the XXY condition so produced. (15 min.) James W. Mavor, Union College.
- 133. The effects of X-rays on the albino rat. (15 min.) Frank Blair Hanson, Washington University.
- 134. The effect of alcohol fumes on three generations of rats. (20 min.) Frank Blair Hanson.
- \*135. Results of breeding potato beetles under changed environmental conditions. (15 min.) A. W. Bellamy, University of Chicago.
- 136. Inheritance of weight in poultry. (15 min.) Henry G. May, Rhode Island Agricultural Experiment Station.
- 137. A lethal type in mice, which may live for a few days after birth. (5 min.) J. A. Detlefsen, Wistar Institute.
- 138. Linkage studies in mice. (Lantern; 5 min.) J. A. Detlefsen, Wistar Institute.
- 139. The heredity of the "white hooded" albino rat. (10 min.) Dorothy R. Stewart, Washington University. (Introduced by Frank Blair Hanson.)
- 140. Recent studies on the relation of metabolism to sex. Oscar Riddle, Carnegie Station for Experimental Evolution.
- \*141. Further studies of the rate of mutation in the bar series of *Drosophila*. Charles Zeleny, University of Illinois.
- 142. The measurement of mutation frequency made practicable. H. J. Muller, University of Texas.
- \*143. Sex-linked inheritance in the teleost, *Platyopocilus maculatus* Gunth. A. W. Bellamy, The University of Chicago.
- 144. Further control of sex in a species of Cladocera. (17 min.) Arthur M. Banta and L. A. Brown, Station for Experimental Evolution.
- 145. A new mutation in *Daphnia longispina* and its inheritance. (10 min.) Arthur M. Banta.
- 146.<sup>1</sup> The translocation of a section of Chromosome-II upon Chromosome-III in *Drosophila*. C. B. Bridges. (Introduced by T. H. Morgan.)

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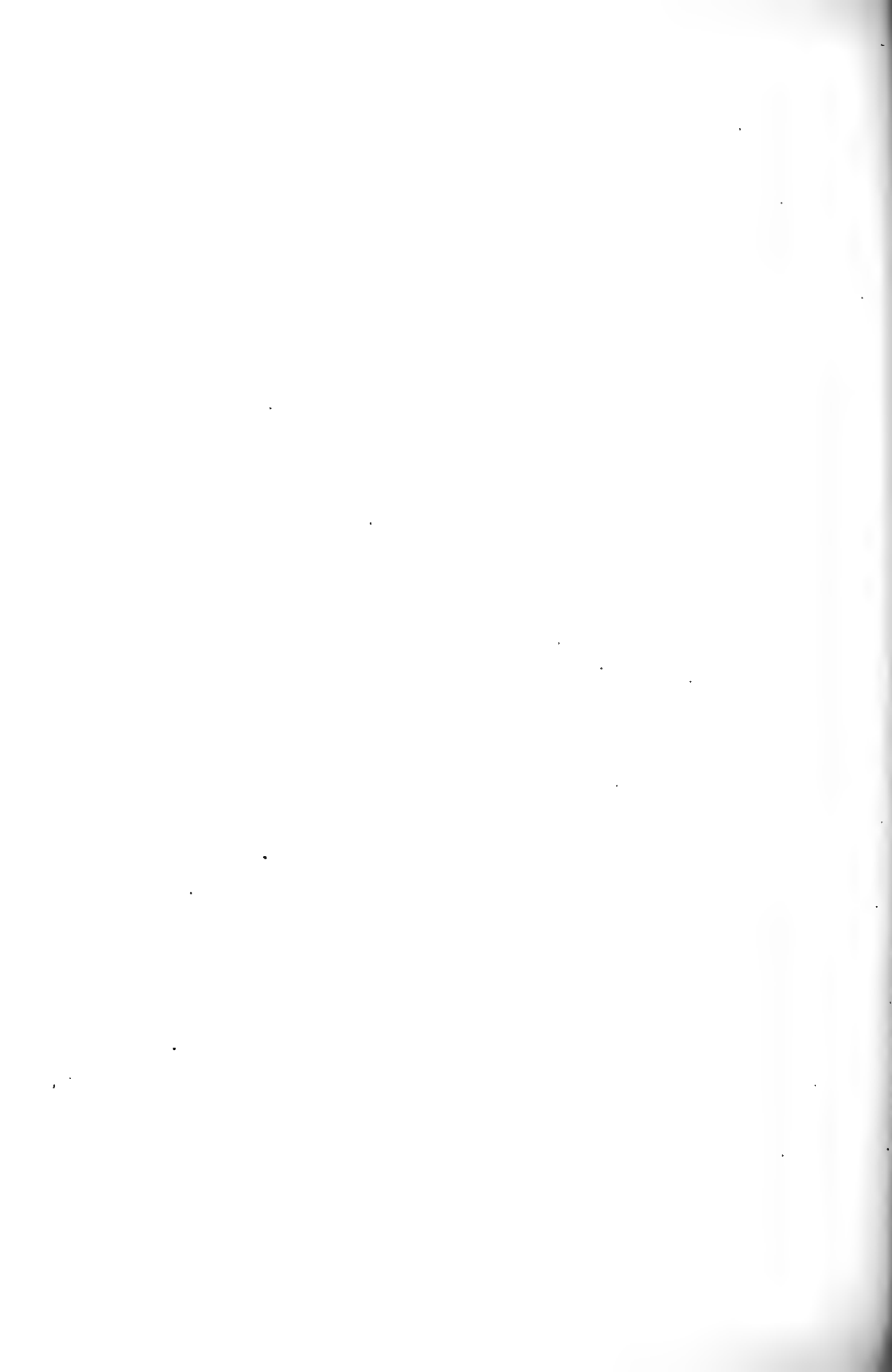
<sup>1</sup> See later abstracts.

JOINT SYMPOSIUM WITH THE AMERICAN SOCIETY OF NATURALISTS  
AND THE ECOLOGICAL SOCIETY OF AMERICA

147. Geographical distribution of certain New England mammals. Glover M. Allen.
  148. Ecological aspects of bird distribution in tropical Africa. James P. Chapin.
  149. Reptile distribution of East and West Indies contrasted. Thomas Barbour.
  150. Distribution of the Amphibia. E. R. Dunn.
  151. Andean and Transandean fishes, their nature and origin. C. H. Eigenmann.
  152. Geographical distribution of land mollusks. H. A. Pilsbry.
  153. Geographical distribution of the Onychophora. C. T. Brues.
  154. Geographical distribution of insects. P. P. Calvert.
  155. Some points in the distribution of New England echinoderms. H. L. Clark.
- (These papers will be published in the *American Naturalist*.)
156. The life-cycle of the Protozoa, C. A. Kofoid, retiring President of the Society and retiring Vice-President of Section F., University of California. (To be published in *Science*.)

EXHIBITS

1. Three microscopic preparations to show the application of the Bielchowsky-Paton method to the nervous system of the earthworm. W. M. Smallwood, Syracuse University.
2. A demonstration of early stages in the establishment of neuromuscular connections. H. V. Neal, Tufts College.
3. Families of butterflies from olive-green and blue-green mutant caterpillars. John H. Gerould, Dartmouth College.
4. Reprints of recent biological studies by Russian biologists. H. J. Muller, University of Texas.
5. A living sphenedon. C. C. Nutting, University of Iowa.
6. Manuscript annotated catalog of the distribution of common invertebrates of the Woods Hole littoral. Manuscript to be deposited with the Bureau of Fisheries and carbon copies distributed by them to representative libraries. W. C. Allee, University of Chicago.
7. Specimens illustrating variation in coat-color of mice of the genus *Peromyscus*. H. H. Collins, University of Pittsburgh.
8. Nine drawings of the cilia of *Scyphidia*. Ruth Jane Ball, University of Vermont.



# ABSTRACTS

## A. PARASITOLOGY

1. *Development of a Myxosporidian, Myxosoma Catostomi nov. spec.* R. Kudo, University of Illinois.

As to the nuclear changes during the course of development of tissue-infecting Myxosporidia, observations of various authors differ greatly. In the trophozoites of *Myxosoma catostomi* which was found to produce a conspicuous tumor in the myotomes of a common sucker, one distinguishes two kinds of nuclei: vegetative and generative. The vegetative nucleus divides by amitosis and further forms generative nuclei by a simple division. In this latter division, the vegetative nucleus gives off a large amount of chromatin material contained in the nucleolus to the newly formed generative nucleus. The generative nucleus becomes surrounded by an island of cytoplasm and grows into a pansporoblast. Gametogony does not occur. The nucleus of the pansporoblast undergoes a heteropolar division. Two nuclei which are produced by a division of the small nucleus become the trophic nuclei of the developing pansporoblast, while the large one divides repeatedly by a primitive mitosis into twelve nuclei which ultimately construct two spores. During the divisions, the latter nuclei throw off large amount of their plasmosome from the nucleoli. This substance forms the spore membrane. Autoinfection by mature spores probably occurs.

2. *Spirochaeta eurygyrata.* M. J. HOGUE, North Carolina College for Women.

*Spirochaeta eurygyrata* was cultured from the liquid stool of a person suffering from chronic diarrhea. Two pure lines were isolated and studied. Locke-egg, ova mucoid, ox-bile salts and sodium chloride-serum water media were used for the cultivation of this organism. On all these media it has lived for over a year.

*Spirochaeta eurygyrata* varies in length from 4-56 microns. Both its ends are rounded. It divides by transverse division which may be equal or unequal. Iron haematoxylin, Giemsa's stain, Cross' stain and weak carbolfuchsin were used with good results. Vital stains did not color the organism. With dark field illumination cross bars were seen in dead spirochaetes but were not seen in the living active forms. Attempts to inoculate kittens by feeding them *Spirochaeta eurygyrata* were not successful. An examination was made of 212 stools from 127 patients but *Spirochaeta eurygyrata* was not present in any of them. It is considered an intestinal parasite of rather rare occurrence in this country.

3. *A new liver fluke from the monkey.* HORACE W. STUNKARD, New York University.

While making parasitological examinations of animals that had died in Bronx Park, New York, I discovered several trematodes in the liver of a white face sapajou monkey, *Cebus apella*, from British Guiana, South America. These worms belong to the subfamily Dicrocoelinae Looss, but can not be assigned to any existing genus.

A new genus *Hepatotrema* is erected to contain the species which is named *H. cebi*. Specimens are 7 to 10 mm. in length and 0.5 to 0.75 mm. in width. The oral sucker averages 0.26 mm. in diameter, the pharynx 0.1 mm. in diameter and the alimentary tract bifurcates above the genital pore. The acetabulum is about one ninth of the body length from the anterior end and averages 0.24 mm. in diameter. The genital pore is midway between the suckers and the cirrus sac extends almost to the acetabulum. The testes lie one behind the other, the caudal testis one third of the body length from the anterior end, the cephalic testis slightly anterior to it. They are lobed, about 0.75 mm. in length and 0.43 mm. in width. The ovary is lobed, slightly anterior to the middle of the body, about 0.32 mm. in diameter. The vitellaria are asymmetrical, on the left side of the body extending from the middle about one half the distance to the posterior end. The caudal half of the worm is filled with uterine coils arranged in a descending and an ascending column. Eggs numerous, 19 to 27 microns.

4. *Observations on an acquired immunity to a metazoan parasite.* LESLIE B. AREY, Northwestern University Medical School.

Immunity to glochidial parasities (e.g., *Lampsilis luteola*) may be acquired by fish (e.g., black bass) in two to five or more infections.

Fish that become thoroughly immune at the second or third infection slough the attached glochidia rather promptly within forty-eight to seventy-two hours.

Fish that require four or more infections acquire an ill-defined immunity and glochidia are lost progressively over several to many days.

Light infections are practically as effective as heavy dosages in producing immunity, although there is apparently a quantitative difference when the spread is extreme. The number of infections seems to be more important than the degree.

The permanency of acquired immunity remains to be proved, yet there are miscellaneous records which indicate that it lasts at least one year.

5. *Variations in Euglenamorpho hegneri, n.g., n. sp., from the intestine of Tadpoles.* D. H. WENRICH, University of Pennsylvania.

This new flagellate, discovered independently by Hegner and the writer, has already been mentioned in "Science" by Hegner. The writer has found two varieties, one green and the other colorless, or nearly so. The body of the green variety is sub-cylindrical, rounded posteriorly with average dimensions of 45 by 5 microns. It has a bright green color, a red stigma, vacuolar and pharyngeal apparatus and spiral surface striations characteristic of *Euglena* but has three equal-length flagella. The laterally placed nucleus is usually compact with the caryosome obscured. The body is filled with flattened oval "corpuscles" about 2.4 by 1.4 microns. Each of the three flagella has near its origin a swelling which stains intensely with haematoxylin.

The other variety has the following characteristics: body colorless or slightly greenish, conical in shape; stigma lacking, swelling on roots of the flagella lacking; nucleus expanded with caryosome prominent, central in position, in diameter nearly equalling that of the body; surface striations variable, prominent to absent, longitudinal to sharply spiral; flagella variable in number, most having either four or six.



In prepared slides no stages in division of the green variety were discovered but mitosis and amitosis were found for the other. In hanging-drop cultures the green variety multiplied but the colorless one did not. In their typical forms the two varieties are different enough to belong to different species but study of prepared slides has revealed many intermediate conditions which indicate that the green form may transform in to the colorless one.

6. *Diphyllobothrium parvum*. THOMAS BYRD MAGATH, Mayo Clinic.

In 1898, a patient, Syrian ae. 37, of Dr. Ramsay in Tasmania passed a tapeworm which was sent by Dr. Elkington to Prof. J. W. W. Stephens who described it as "*Dibothriocephalus parvus*," in 1908. The head was unknown.

In 1920, a patient in the Clinic passed, following treatment, a worm which was identified as *Diphyllobothrium parvum*. The patient was a female ae. 49, born near Warsaw and altho she came to America at the age of one, she has never returned to Europe. She is known to have had the worm for at least six years. Her home has been for some time in Chicago. This is the second record of infection by this worm and a morphological description is offered.

7. *Notes on Acanthocephala from Japan*. H. J. VAN CLEAVE, University of Illinois.

There have been no published records concerning the acanthocephalan fauna of Japan. A collection received from Professor S. Goto contains a new species of the genus *Arhythmorhynchus*, larval representatives of the genus *Centrorhynchus*, and three new species of the genus *Acanthocephalus*. Of these last, one species is from the intestine of an eel while the remaining two are from amphibians.

The amphibian parasites are of especial biological interest because of evidences which they present of a close parallelism between members of the genus *Acanthocephalus* infesting European and Japanese Amphibia. A species from Japanese urodeles bears strong resemblances to *Ac. falcatus* of European urodeles but displays unquestionable specific distinction from it. In similar manner specimens of the same genus from Japanese Anura closely parallel the European species *Ac. ranæ*.

The parallelism involves not only sizes of critical structures such as proboscis hooks and embryos but also extends to the tendency for variability in numbers of hooks which is much more pronounced in the anuran parasites than in the species from urodeles.

8. *The relations between the food of frog and toad tadpoles and their intestinal protozoa*.

R. W. HEGNER, Johns Hopkins University.

During the early stages of metamorphosis of green frog tadpoles Opalinae are numerous in the rectum but not in the intestine. During intermediate stages the Opalinae appear to migrate into the intestine and in late stages and in young and old frogs they disappear entirely. Infection of the green frog with *Nyctotherus*, *Trichomonas* and *Hexamitus* is continuous from tadpole to adult; and infection of the leopard frog and toad with these intestinal protozoa as well as with *Opalina* is also continuous from tadpole to adult. The incidence, distribution and numbers of these protozoa become modified under experimental conditions as follows. *Opalina* is rarely present in tadpoles after these are kept without food for two weeks; *Nyctotherus* disappears usually in about one week; but *Hexamitus* persists in considerable

numbers for a long period. Tadpoles of the green frog were fed on various glandular substances including desiccated thyroid, thymus, ovaries, prostate, pituitary, suprarenal and orchic. The most striking results were obtained with thyroid, which changed considerably the distribution and numbers of protozoa in the digestive tract. In heavily infected tadpoles Opalinae were most numerous in the rectum and only few in number in the intestine. After a diet of thyroid for 4 or 5 days the Opalinae were found to have migrated from the rectum to the intestine. Such a migration also occurs during normal metamorphosis and hence the conclusion is reached that the change is due not to the thyroid but to the rapid metamorphosis of the tadpoles brought about by the thyroid diet.

9. *The use of the pure culture and the guinea pig in biological studies of parasitic nematodes.* B. H. RANSOM, U. S. Bureau of Animal Industry.

The growing of pure cultures of bacteria and the use of the guinea pig as an experimental animal are commonplaces of bacteriology. The "pure" culture and the guinea pig, however, have been but little used in biological investigations on parasitic worms. Their usefulness in this field are nevertheless very great. Large numbers of larvae of various parasitic nematodes can readily be reared to the infective stage in "pure" cultures containing only one species of nematode. When introduced into the guinea pig the infective stages of parasitic nematodes, even in the case of species that in nature appear to be narrowly limited in their choice of hosts, often establish themselves and undergo developmental changes and migrations similar to those that occur in the usual hosts. The "pure" culture and the guinea pig in the writer's experience have been very useful not only in investigations on the life history of Ascaridae but are continuing to be serviceable in life history studies of other monoxenous nematodes. For example, it has been found that the larvae of the gapeworm (*Syngamus trachealis*) in the infective stages will migrate to the lungs of the guinea pig and undergo development there to a later stage, that the infective larvae of certain strongyles of the horse will establish themselves in the wall of the intestine of the guinea pig; and that the larvae of the stomach worm of ruminants (*Haemonchus contortus*), when introduced into the guinea pig, will continue their development and grow to a considerable size.

10. *Studies on Necator suillus, parasitic in pigs.* JAMES E. ACKERT and FLORENCE K. PAYNE, Kansas State Agricultural College.

Recent hookworm investigations in Trinidad, British West Indies, showed that native pigs were infested with a new parasite, *Necator suillus* Ackert and Payne, 1922. Post mortem examinations of swine and culturing samples of pig feces from various localities indicated that *N. suillus* is of frequent occurrence and wide distribution on the Island. Pigs of all ages were subject to infestation with *N. suillus*, but those five or more months of age showed both a higher percentage of infestation and a large number of hookworms. This may have been due to the custom of severely limiting the pigs' ranges which becoming polluted with the hookworm eggs made ideal culture media for the larvae during the rainy season. Results of several attempts to infect young pigs with infective larvae of the human hookworm, *N. americanus*, administered *per os* and on the skin indicate that *N. americanus* cannot mature in the pig, although a typical case of "ground itch" was produced on a young

pig's body. Morphological studies on live and preserved specimens of human and of pig hookworms, and comparisons with descriptions of other species of *Necator* indicate that there are now four known species of this genus viz., *N. americanus* Stiles, 1902; *N. exilidens* Cummins, 1912; *N. congolensis* Gedoelst, 1916; and *N. suillus* Ackert and Payne, 1922.

11. *The life-history and development of the mite, Myobia musculi, Schrank.* HOWARD E. ENDERS, Purdue University.

Specimens of the mites were collected from the heads and bodies of the brown rat, *Mus norvegicus*, and from white rats and house mice. The legs of the mites are very short and thick, but the first pair is modified remarkably into devices fitted to clasp the hairs of the host. Its eggs are attached to hairs, very close to the skin. When the young emerge from the eggs they possess six legs, of which the first pair possesses the characteristic clasping devices. By means of these claspers they attach themselves between two hairs in such relation that the beak is thrust deeply into the skin of the host. Two molts succeed one another and lead to the development of the adult individual upon the skin of the host animal.

Early descriptions account for the passage of the nymph into the hair follicles where it was said to feed and to transform into the adult condition.

*Myobia musculi* was reported by Osborn from the heads of mice.

12. *The effect of carbon tetrachloride on intestinal protozoa. A preliminary note.* M. J. HOGUE and C. VAN WINKLE.

The effect of carbon tetrachloride has been tried on cultures of *Trichomonas hominis*, *Embadoomonas intestinalis* and *Spirochaeta eurygyrata*. In all cases the organisms were killed by the addition of one small drop of the drug to a cover glass preparation.

Kittens infected with giardia and spirochaetes were given one cc. of carbon tetrachloride. The giardia cysts usually disappeared from the stools for 6 or 7 days following the treatment. They would then reappear. Another dose of carbon tetrachloride would cause them to again disappear for a few days after which they would reappear. Spirochaetes appeared at irregular intervals in the stools of the kittens in spite of the treatment with carbon tetrachloride.

A child infected with *Trichomonas hominis* and *Endamoeba nana* is being treated with carbon tetrachloride. *Endamoeba nana* cysts appeared in the stools in large numbers the day after the administration of one cc. of carbon tetrachloride. Since then they have been absent. The *Trichomonas hominis* still appear in cultures of sodium chloride-serum water. The patient is under observation and is still receiving treatment.

13. *A new genus of trematodes from the Eastern painted turtle.* HORACE W. STUNKARD, New York University.

A very small blood fluke was discovered in the arteries of *Chrysemys picta* taken in New York and New Jersey. These trematodes belong to a new genus in the subfamily Spirochinae and constitute a third genus in that group. To them the name *Haematotrema parvum* is given. Sexually mature worms measure 0.75 to 2 mm. in length and 0.05 to 0.12 mm. in width. They may be briefly described as

follows: Exceedingly small and slender monostomes with delicate body which tapers towards both ends. Oral sucker large, elongate, protruding; relatively long esophagus and esophageal glands not strongly developed; intestinal crura sinuous, extending almost to the posterior end of the body. Testes lobed, four or five in number, situated in the anterior part of the posterior half of the body; seminal vesicle between testes and cirrus sac; genital pore below the cecum of the left side, one fourth of the body length from the posterior end. Ovary lobed, on the right side, at or slightly anterior to the level of the genital pore; vitellaria envelop the ceca throughout their length and fill the intercecal area anterior to the testes and posterior to the ootype; seminal receptacle and Laurer's canal present; uterus short; eggs very large, operculate, discharged singly.

14. *Cross-infection of Muscoid flies with Herpetomonas.* ELERY R. BECKER, Johns Hopkins University. (Introduced by W. H. Taliaferro.)

With two or three exceptions, the workers who have investigated the flagellate parasites of non-biting flies have been influenced by the theory that each host species is parasitized by a distinct species of flagellate, with the result that the literature contains descriptions of flagellates hardly morphologically distinguishable, such as *Herpetomonas muscae-domesticae*, *H. sarcophagae*, *H. calliphorae*, *H. luciliae*, etc.

Examination of the intestinal tracts of a number of North American muscoid flies indicated that they may be infected with a *Herpetomonas* which shows no more morphological variations in hosts of different species than it may show in different hosts of the same species. In order to determine whether or not there might be physiological variations which rendered these flagellates, which apparently belonged to the same species, specific for their hosts, a number of cross infection experiments were undertaken. "Clean" flies of the following species were bred in the laboratory: *Musca domestica*, *Lucilia sericata*, *Phormia regina*, *Chrysomya macellaria*, *Calliphora erythrocephala*, *Sarcophaga bullata*, and *Sarcophaga securifera*. In none of these species could there be found any hereditary transmission of a *Herpetomonas* infection. "Clean" flies belonging to each of these six species were then fed the contents of the intestine of infected "wild" flies of the other species. In no instance was there failure to infect. A number of flies of each species approximately equal to the number of flies used in each experiment were kept under the same conditions as controls. In no case did the controls become infected.

The results of the experiments prove that the herpetomonad parasites of the common muscoid flies are non-specific for their hosts.

15. *Interactions between protoplasmic masses as a means of determining physiological variations in Arcella.* B. D. REYNOLDS, Johns Hopkins University. (Introduced by R. W. Hegner.)

Kepler and Reynolds (in press) have shown that, if a pseudo-pod is severed from *Diffugia* and the two parts, i.e., the organism and the severed fragment, are placed near each other, restitution of the whole is usually accomplished by fusion. This phenomenon has been observed to hold true for several genera of Rhizopods. In the course of further investigations the writer has found, in attempting to cause fusion between pseudopods that had been severed from one specimen and other individuals of the same species, that frequently instead of fusion taking place, there

would be a violent contraction of the involved protoplasm, accompanied by a shattering of the fragment, and sometimes also the contracting pseudopods of the organism, after which the animal would move away leaving the bead-like masses of protoplasm behind. By applying this principle it is possible to determine that:

(a) Under similar environmental conditions cross-fusions between protoplasmic masses from specimens belonging to the same pure line cease after about the 22nd generation, i.e. shattering of protoplasm occurs.

(b) By slightly altering the environment in one pure line, this phenomenon can be greatly hastened—the time required for negative reaction being reduced to five or six generations.

(c) By keeping both lines in an identical environment (the same concavity) cross-fusion will continue through at least 150 generations. (This experiment is still in progress).

(d) After specimens from different branches of the same pure line have become negative to each other, cross-fusion can be induced again by placing them in the same concavity; the length of time required to bring this about apparently depending upon how long the negative state has persisted.

16. *Variations in Taenia saginata, the "beef" tapeworm of man.* FRANKLIN D. BARKER, University of Nebraska.

A comparative study of 300 mature proglottids from 30 specimens of *Taenia saginata* reveals frequent and marked variations in the genitalia. Variations in the shape and size of the ovaries and vitellarium were especially noticeable. We have found proglottids in which was duplicated the condition as described in *Taenia philippina* by Garrison, 1907 and as described in *Taenia confusa* by Chandler, 1920. We have found ovaries and vitellaria very similar to those described by Guyer, 1898 for *Taenia confusa*. We believe that the variations in *Taenia saginata* are more extensive than heretofore considered.

A comparative study of gravid proglottids and eggs is now in progress.

17. *The chromosomes in Ascarus lumbricoides of man.* FRANKLIN D. BARKER, University of Nebraska.

A large number of eggs from three fertile female acarids of man were studied. The number of chromosomes was found to be forty-eight, the size and shape of chromosomes and the chromosome complex was the same as that described by Boveri, Carnoy, Fürst and Edwards for *Ascaris suum* (*Ascaris lumbricoides* from the pig).

The recent work of Bakker, 1921 seems, from the histological standpoint to establish the identity of *Ascaris lumbricoides* of man and *Ascaris suum* of pig.

Our work of the chromosomes would substantiate this conclusion.

## B. CYTOLOGY AND HISTOLOGY

18. *Cellular elements in the perivisceral fluid of the Echinodermata.* JAMES E. KINDRED, Western Reserve University.

Leucocytes alone are present in the perivisceral fluid of the Asteroidea studied; leucocytes, colorless amebocytes and vibratile corpuscles in the perivisceral fluid of the Ophiuroidea; leucocytes, colorless amebocytes, pigmented amebocytes and

vibratile corpuscles in the Echinoidea; and leucocytes, colorless amebocytes and hemocytes in the perivisceral fluid of the Holothuroidea.

The leucocytes found in the perivisceral fluid of the representatives of the classes of Echinodermata studied, are characterized morphologically, by flap-like processes of the ectoplasm and a granular endoplasm containing vacuoles. The content of the vacuoles is acid in reaction. Physiologically the leucocytes are phagocytic, thrombocytic, scleroblastic and fibroblastic.

Amebocytes containing red pigment vacuoles are characteristic of the Echinoidea, and from their concentration in the regions of the ampullae of the respiratory tube feet, they are assumed to be associated with respiration.

Different concentrations of hemoglobin are present in the non-ameboid hemocyte characteristic of the Holothuroidea. The hemocytes of *Stichopus* are very minute and flagellated, while those of *Cucumaria* are large and have a prominent nucleus.

A saturated solution of methylene blue in seawater stains the vacuoles of all the leucocytes. The colorless amebocytes of the Holothuroidea are the only other cells of the perivisceral fluid which are affected by this stain.

Neutral red in seawater stains the vacuoles of the leucocytes a reddish orange. The vacuoles of the colorless amebocytes of the Holothuroidea are stained a cherry red with neutral red.

19. *The application of the Bielchowsky-Paton method to the nervous system of the earthworm.* (Lantern.) W. M. SMALLWOOD, Syracuse University.

Thus far this silver-gold method has reacted only on the nerve-fibers in the several organs of the earthworm, and does not enable me to trace the different kinds of fibers in the nerve trunks to the ventral ganglia. It, however, reveals that the distinction between efferent sensory and motor nerves is not as sharp as previously stated, and that the nervous system is very much more extensive and comprehensive in its relation to the various organs than hitherto reported. There is a profuse plexus in the muscles, nephridia, peritoneum, blood vessels, and intestinal wall in which the nerve fibers that end in muscles are continuous with those that end in the cells of the nephridia. This plexus seems to be continuous from the free-nerve endings in the epidermis to the free-nerve endings in the intestine. Nerves have been traced from the longitudinal muscles to nephridia and from the muscles in the intestinal wall to their free-ending between the digestive epithelial cells under the oil-immersion lens. Specific end-organs in the muscles and nephridia are shown by this method.

20. *Luteal cells in the gonads of the Phalarope.* (By title.) HARRY B. YOCOM, University of Oregon.

A histological study has been made of the gonads of the Northern Phalarope (*Phalaropus lobatus*, Linn.) to determine whether or not there exists any correlation between the presence of luteal cells and the color differences of the two sexes. In this group of birds the female is more brilliantly colored than the male, especially in the breeding plumage.

Material was collected during both the spring and fall migrations. The ovaries taken at both times show the characteristic packets of luteal cells in the tissue around the oocyte follicles. They seem to be slightly more conspicuous in the ovaries

of the birds taken in the Fall at which time the females have lost part of their bright colors and the two sexes are more nearly alike. A study of the testes failed to reveal any packets of luteal cells and differential stains did not bring out any cells which might unmistakably be considered luteal cells.

This evidence would indicate, in the Phalarope at least, that the suppression of color in the male is due to some factor other than the presence of luteal cells in the testis. The question arises whether or not we are dealing with the same problem in considering the coloring of birds such as the Phalarope as in considering hen-feathering in fowls where there is not only a color difference but a structural difference in the feathers as well.

21. *The nature of the division of neuroblastic cells in the regenerating spinal cords of Amphibian larvae.* DAVENPORT HOOKER, University of Pittsburgh School of Medicine.

Injuries to the spinal cord of Amphibian larvae (*Amblystoma* and *Rana*) produce proliferation of cells of neuronal type during regeneration. This proliferation is accomplished by mitotic division of indifferent cells situated in the mantle layer, usually close to the ependyma. Such divisions are found in experimental animals of all ages up to and including metamorphosis. In the cords of young normal embryos many such mitoses are found, but they are rare in older larvae.

The identification of these cells as of neuronal potentiality rests upon their cytological characters, their position and the known increase in number of neurons during regeneration. A neuroblast may be regarded as a neuronal cyton only when it has processes. Such cells do not divide. The indifferent neuroblastic cells provide the additional neuronal elements by mitotic division.

Careful examination fails to elicit any evidence of the presence of amitotic cell divisions in the spinal cord in any of the larvae studied. In many instances two cells lying in close apposition present figures which so strikingly simulate amitoses that it is at times difficult to establish their real nature.

These observations are of interest in connection with the work of Agduhr, 1920, who reports the presence of amitotic divisions of neuroblasts in young mammals following exercises. The observations reported above would make it appear probable that Agduhr has misinterpreted his material.

22. *Sources of nutriment during the metamorphosis of fresh-water mussels.* LESLIE B. AREY, Northwestern University Medical School.

The nutriment necessary for the metamorphosis of a larval mussel while parasitic on its fish-host is gained from three distinct sources:

1. The bitten host-tissue is utilized by extracellular digestion in the mantle cavity and by phagocytic ingestion and intracellular digestion within the mantle cells.

2. Following this period of tissue reduction, the larval adductor muscle breaks down and is transported bit by bit by amoeboid cells to the mantle which receives the fragments, reduces them to finer particles, and converts them to available food.

3. Fluid interchange between host and parasite undoubtedly occurs. This, however, is not mediated through the vessels clasped by the valves during attachment; on the contrary, it is by general diffusion of the tissue juices which bathe the encysted glochidium.

23. *The typical form of polyhedral cells in plant parenchyma and in human epithelium.* (Lantern.) FREDERIC T. LEWIS (introduced by Herbert W. Rand), Harvard Medical School.

The typical form of cells, when subjected to the mutual pressure of similar cells on all sides, has apparently never been determined objectively. Usually they are described as rhombic dodecahedra, on mathematical grounds, and because they tend to be hexagonal in both vertical and transverse sections. Kieser (1815) considered that in vegetable parenchyma the rhombic dodecahedra are truncate above and below, thus having the top, base, and two sides hexagonal, and the remaining eight sides quadrilateral. Plateau, Kelvin, and others postulated that space would be divided with minimal partitional area by bodies with 14 sides, 8 hexagonal and 6 quadrilateral—*tetrakaidecahedra*; but Thompson (1917) concludes that "very probably . . . the rhombic dodecahedral configuration, even under perfectly symmetrical conditions, is generally assumed." This we find not to be the case. In the pith of *Sambucus canadensis* the contacts of 100 cells were counted, with the result here tabulated, the lower row of figures showing the number of cells having the number of surfaces indicated by the figure above.

No. of surfaces	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
No. of cells	1	1	2	0	2	8	9	20	16	19	10	2	3	6	1

Average number of surfaces, 13.96. Forty cells, reconstructed in wax from serial sections, vary greatly, but usually have hexagonal surfaces above and below, and are hexagonal in vertical section, with an equatorial ridge. These models (made by Ethel S. Lewis) will be shown, and interpreted as approximate *tetrakaidecahedra*. Examination of the smaller cells of human stratified epithelium is more difficult. A preliminary study of ten cells yield an average of 13.8 contacts. Models will be shown.

#### C. UNCLASSIFIED

24. *Fiji-New Zealand expedition from the University of Iowa.* (60 min., lantern.) C. C. NUTTING, University of Iowa.

A popular account of the experiences of the recent Fiji-New Zealand expedition, including contacts with the natives as well as with the animals with which the expedition was immediately concerned. Among other specimens collected are four living sphenodons one of which will be exhibited. The entire account will have a decided zoological "slant."

25. *Observations of biological science in Russia.* H. J. MULLER, University of Texas.

In a visit to Russia during the past summer the author found the biological scientists far more active than is generally supposed. They are in the main gathered together in a series of large state research institutions. Among these is Koltzof's "Institute of Experimental Biology," with laboratories in Moscow and two stations in the country, for work in hydrobiology and genetics, respectively. At the latter, under Lebedof, work on fowl and rodents, including crossing-over studies, and studies of enzymes genetics is being done. Lazarif's well equipped "Institute of Physics and Bio-physics" with a staff of about 50 scientists, is developing and testing



out important theories of the physico-chemical mechanism of excitation and conduction. There are various other interesting biological institutes in Moscow and Petrograd, but space does not permit mention of their work here, except to suggest their scope by reference to a few salient researches, such as the cultivation of the typhus germ (Barikin and Krich); the development of micro-methods in enzyme determination (Bach); the work of Ivanov on sperm; of Vavilov on cereal variation; of Martsinovskiy and others on malaria organisms, Leishman-Donovan bodies, etc. Experimental work is also being done in the universities: for instance, Isaiev's work on interspecific transplantation in hydra, and Nicolaeva's on chromosome aberrations.

Although living in Russia is still subject to great hardships, conditions are improving and the scientists are hopeful. Their most crying need, in their work, is for communication with the Western world—periodicals, books, and exchange of visits.

26. *The proper wording of titles of scientific articles.* E. W. GUDGER, American Museum of Natural History.

Experience in bringing together into a complex analytical subject catalogue the 43,000 titles of fish literature embraced in the "Bibliography of Fishes," now being issued by the American Museum of Natural History, emphasizes the need for better and more complete titles for scientific articles.

Our work has showed that thousands upon thousands of titles give either no idea or else a faulty one of their contents, and the necessity for clearing them up by actually seeing the articles has added many months of time and many hundreds of dollars to the cost of completing this subject-catalogue.

The title of a scientific article should state as accurately and clearly as possible just what the article treats of, anatomy, embryology, physiology, etc.; or if it treats of some obscure structure or function, that structure or function should be named; then the scientific and the common name of the beast should be given, and its class named, i.e., fish, amphibian, bird, etc.

Scientific articles are written to diffuse knowledge, but the intermediates through whom this diffusion is effected are bibliographers and librarians, and their helping hands are badly tied by the insufficient and misleading titles of a large number of scientific books and articles.

27. *Variations in coat-color within a single subspecies of mice of the genus Peromyscus.* (Lantern.) H. H. COLLINS, University of Pittsburgh. (Introduced by E. F. Adolph.)

A study of the various factors which determine the color differences within a single subspecies, involving both field studies and laboratory experiments has shown the following: (1) Changes in color due to fading and abrasion are slight. The color differences observed within a subspecies and described as "buff" or "light" and "dark" or "gray" phases cannot be ascribed to seasonal changes through which any individual may pass during the year. (2) These color phases are not due to environmental differences, acting during the lifetime of a single individual or even within a few generations. Individuals representing both extreme phases have been taken in the same locality. Furthermore their descendants, reared in captivity under identical environmental conditions for several generations, have bred true to

type. (3) The buff or light phases do not, as has been suggested, represent old as contrasted with younger adults. Specimens kept in captivity for a period of nearly six years have not undergone any such change. (4) The differences in color are mainly genetic in character. The offspring of buff and dark parents are likewise buff and dark and moreover these differences are apparent soon after birth. When the buff and dark strains are crossed, the offspring are of an intermediate character. The  $F_2$  generation is also intermediate in coloration.

#### D. ENTOMOLOGY

28. *Afferent and efferent pathways in Dendroides.* WILLIAM A. HILTON, Cornell University.

The larva of this small beetle has many advantages for study. The flat body and nearly transparent tissues make it possible to follow all the nerves in abdominal segments.

The single lateral branch from each ganglion is evidently mixed but parts of it could be clearly analyzed into afferent and efferent components.

Many fibers of the dorsal sensory nerve pass to the ganglion above before terminating. The results of experimental stimuli to body segments confirm this distribution. The sensory branches of each ganglion have no wider distribution than the segment to which their ganglion belongs. This is also probably true of motor branches with the possible exception of some of the nerve strands which supply the heart.

So far as studied, sensory endings were by means of bipolar cells ending chiefly in hollow hairs. Similar cells were seen in association with muscles, perhaps for the reception of the muscular sense. The true motor endings were clearly of another type. Unipolar or bipolar cells located in the ganglia on the same or opposite side send out processes to muscles. The branches from motor nerves run out parallel to the muscle fibers and terminate as long twigs or decided end brushes. The number of sensory nerve cells in a segment was easily determined. This number was compared with the fibrillae of the nerve trunk and some suggestions made as to the constitution of fiber and fibril.

29. *Ant larvae.* (Lantern.) W. M. WHEELER, Harvard University, and G. C. WHEELER, Syracuse University.

While the classification of insect larvae has made notable advances since the days of Linnaeus, ant larvae have been generally neglected. This neglect has been due to lack of interest rather than to such difficulties in rearing and determining material as one encounters among other groups. We have studied the larvae of 125 of the 268 genera of ants with the result that we have been able to prepare keys for the subfamilies, tribes and genera. It has also been possible to clear up doubts which myrmecologists have entertained concerning the taxonomic affinities of certain genera (e.g. *Gigantiops* and *Leptanilla*).

The larvae of the Dorylinae and Cerapachyinae are elongate, slender, cylindrical, and nearly straight, with the mouth-parts reduced. The Ponerine larvae with their swollen bodies and stout mandibles are most like the larvae of the social wasps and probably represent the ancestral type. The trophorhinium, or triturating apparatus

elaborated on the mouth-parts, attains its maximum development in this subfamily. The larvae of the four Pseudomyrmine genera are straight and subcylindrical and present a striking assemblage of unusual structures, such as exudatoria and the trophothylax, or larval "feed-bag." The larvae of the Formacinae resemble those of the Ponerinae, but the mouth-parts are more specialized. The Myrmicinae are a heterogeneous group. The plump, chunky Dolichoderine larvae with ventrally placed head and vestigial mouthparts are the most specialized.

30. *Further observations on the digestive system of the periodical cicada.* CHARLES W. HARGITT and L. M. HICKERNELL, Syracuse University.

The morphology of the digestive system in the several embryonic stages is described, and the developmental history of the parts of the system outlined. None of the findings warrant any change of view in interpretations made upon adult material in an earlier paper. The embryonic material throws much light upon the functional significance of parts of the system which up to this time have not been completely understood.

Attention has also been directed to matters of physiology and correlated phases of behavior, especially those of food taking as related to adult life and reproduction. This will be shown to have relations to the anomalous aspects of larval life, among them the excessive development of the "fat-body" in this insect. Comparison is made with similar provision in several other organisms, both among insects and vertebrates, going to show adaptation to the stress involved in the growth and rapid maturation of the sex products, and the laborious deposition of the eggs, all of which is comprised within the brief period of two or three weeks of adult life. Brief notice is taken of some recent views touching the problems concerned.

## E. EMBRYOLOGY

31. *Periodicity in the production of sexual cells in marine animals.* B. H. GRAYE, Wabash College.

The experience of members of the teaching staff and of various investigators at Woods Hole indicates that there is a lunar periodicity in the maturing of germ cells in the starfish, the sea urchin and *Cumingia* as well as in *Nereis* and *Chaetopleura* which have been previously described. Notes of various investigators show that *Cumingia tellinoides* sheds its gametes most abundantly at the time of the full moon and possibly at the new moon; i.e. fortnightly maxima.

This periodicity is not readily apparent during the height of the breeding season but comes out clearly toward the end.

32. *Germ cell and germ gland development in male Rana catesbeiana tadpoles.* W. W. SWINGLE, Yale University.

Different races differ in testicular development. Two methods of testis formation occur, direct and indirect, each characteristic of various local races. Eight races were examined, five exhibited indirect, three the direct method of testis formation. The problem has nothing to do with sex determination or sex transformation but is a cycle of degenerative and regenerative processes in testis development-processes

comparable in many respects to the annual cyclical changes of the testis of certain urodeles.

Characteristics of indirect testis formation are as follows: delay in formation of the definitive sex (rete) cords with consequent persistence and thickening of the germinal epithelium—definitive sex cords may not be formed for two years or more in some races; abortive maturation cycle produces spermatocytes and sometimes aberrant spermatids; degeneration of all maturation cells; the lobules of the pro-testis (larval gonad of races exhibiting indirect testis formation) contain residual spermatogonia; definitive sex cords form, and cross tubules grow out to the residual spermatogonia of each pro-testis lobule; part of the residual spermatogonia migrate down the tubules into the sex cords and by rapid multiplication form the anlagen of the definitive testis which develops as a core of sex-cords and residual spermatogonia surrounded by degenerating pro-testis lobules; residual spermatogonia of the pro-testis regenerate the lobules and these also become a part of the definitive testis.

Evidence exists that races with direct testis formation undergo an abortive post-larval sexual cycle culminating in degeneration of the male sex products.

33. *The microdissection of the egg of Cerebratulus.* GEORGE A. BAITSELL, Yale University.

The following results have been obtained from a series of micro-dissection experiments on the eggs of *Cerebratulus*: The egg membrane, which forms when the eggs are placed in sea water, is a great hindrance to microdissection. It can be removed (Chambers, '17) without injuring the eggs by forcing them through a fine meshed cloth. Such eggs appear to be naked, but the presence of a very delicate membrane may be demonstrated with the needle. A prominent germinal vesicle, with a nucleolus, is present in the eggs for several minutes after they are placed in the sea water. It possesses a definite nuclear membrane which is very sticky. This can be shown by bringing the point of the needle in contact with it. Frequently the membrane will stick so tenaciously to the needle that the entire nucleus can be drawn through the surrounding cytoplasm and completely out of the egg. The nuclear membrane can then be removed from the nucleus. In such cases the prominent nucleolus rapidly disappears, and, shortly, the nuclear membrane also fades from view. The rest of the nucleus, however, persists as a transparent, homogeneous body, and it can be shown by microdissection to be a rigid gel, the pieces of which show no tendency to alter their shape in the sea water. The experiments thus far made on the eggs indicate that changes in the viscosity of the cytoplasm occur, but the results obtained do not entirely agree with those of previous investigators.

34. *The presence of copper in Arbacia eggs.* OTTO GLASER, Amherst College.

Copper can be demonstrated in *Arbacia* eggs by a variety of methods. It can be localized directly by means of specific reactions, indirectly by analysis of egg secretions. The quantities involved per cubic centimeter of eggs are seventeen micrograms for unripe ovarian eggs, 175 for ripe shed eggs and 21 for fertilized eggs. The copper is found associated in part with lipolysin; it is also associated with preparations of pancreatin and pepsin. These facts are the basis for certain suggestions regarding the mechanism of oligodynamic action of heavy metals.

35. *Analysis and interpretation of lithium effects in echinid embryos.* J. W. MAC-ARTHUR, University of Toronto.

Characteristic *structural* modifications induced by lithium in embryos of sand-dollar and sea urchins were, as in Herbst's data changes: (1) in general form and relations (degrees of exogastrulation); and (2) in proportion of parts.

For a *physiological* analysis the physiological gradient conception of Child was found most helpful. In high concentrations lithium caused general inhibition of development; in lower concentrations the retardation was rather sharply localized, for growth and mitosis were slowed down or stopped in apical regions (animal pole; ectoderm), but little or not at all diminished in basal regions (vegetal pole; entoderm; mesenchyme). In extreme cases ectoderm was reduced to the vanishing point, while entoderm and mesenchyme underwent a reciprocal hypertrophy at its expense. Significantly, in such types the fore-gut is most increased, and the hind-gut least. Regarding exogastrulation it is suggested that it is almost impossible mechanically for a greatly enlarged entoderm to invaginate into a very small blastocoel already visibly overcrowded by excess of proliferated mesenchyme.

In susceptibility to KNC, etc. normal embryos show a distinct apico-basal gradient (ectoderm > entoderm > mesenchyme), but in lithium-modified embryos clearly this gradient is levelled down and finally quite completely reversed (mesenchyme-entoderm > ectoderm).

*The lithium modifications appear to follow logically and necessarily form the altered metabolic relations of the reversed gradient, just as the normal proportions and relations of parts follow from the usual metabolic relations.*

36. *Cryptorchidism experimentally produced.* CARL R. MOORE, University of Chicago.

If rat or guinea pig testes are replaced in the peritoneal cavity for three to four months (inguinal canals open or closed) the testis is histologically typical of an undescended testis. The germinal epithelium is absent; the small seminiferous tubules contain besides a reticular mass, a single layer of cells usually poorly stained (considered Sertoli cells); the interstitial cell mass is abundant.

If but one testis has been so treated, or if both were replaced in the peritoneal cavity and one subsequently returned to the scrotal sac, the one remaining in the cavity will be typically cryptorchid whereas the scrotal testis is normal.

Seven days after operation degeneration of the testis is evident; at fourteen days well advanced; and at twenty to thirty days the germinal epithelium is usually absent. In the process of degeneration the epithelial cells unite into discrete syncytial masses, similar to giant cells, in the epithelial layer or free in the lumen of the tubule.

If the scrotal sac is everted and fastened in the peritoneal cavity (with testis) a similar degenerate testis is produced. The vas deferens, blood supply, nerve supply and scrotal sac connections are normal.

After considerable degeneration has taken place, the testis will recover and return to normal if replaced in the scrotal sac (data incomplete to date).

Since factors such as infection, greater pressure, severance of blood or nerve supply and scrotal sac connections have been eliminated as causes of degeneration, it is suspected that differential body temperature may be a factor.

57. *Iodine and anuran metamorphosis.* (Lantern.) W. W. SWINGLE, Yale University.

Three groups of thyroidectomized and hypophysectomized *Rana sylvatica* tadpoles were used in the experiment. One group was fed tyrosine; another group was fed tyrosine in which two atoms of iodine had been substituted for two hydrogen atoms of the molecule forming the compound 3-5-diiodotyrosine. The third culture received equivalent quantities of 3-5 dibromtyrosine-tyrosine in which two bromine atoms had been substituted for two hydrogen atoms.

The tyrosine and dibromtyrosine-fed animals showed no evidences of metamorphic change after two months of continuous feeding; conversely the iodotyrosine-fed tadpoles metamorphosed (except for the presence of the tail stump in pituitaryless animals) within twenty days from the date of first feeding.

A similar experiment was carried out on *Rana clamitans* tadpoles with intact thyroid glands. The iodotyrosine-fed animals metamorphosed within twenty days. The tyrosine and dibromtyrosine-fed animals showed no indications of transformation after two months of continuous feeding.

Anuran metamorphosis is dependent upon a peculiar property inherent in the iodine atom when combined in a certain way. The iodine to be effective need not undergo any transformation within the thyroid gland. Thyroid iodine is by far the most effective in accelerating metamorphosis but other forms of iodine can replace it insofar as metamorphosis is concerned.

Bromine has no influence upon metamorphosis and cannot be substituted for iodine.

58. *Iodine and urodele metamorphosis.* W. W. SWINGLE, Yale University.

The following experiments render invalid the conclusion of E. Uhlenhuth that the metamorphosis of urodele larvae is independent of iodine and its compounds and influenced only by the thyroid hormone.

1. The thyroids of axolotls (seven inches long) were extirpated and the animals kept for five months following the operation. They were then injected twice at five day intervals, with strong doses of diiodotyrosine. The animals metamorphose within seventeen days following the first injection. Control thyroidless axolotls injected with equal quantities of pure tyrosine and dibromtyrosine (two atoms of bromine in the molecule) showed no evidences of metamorphosis.

2. Thyroidless axolotls were kept for five months and then injected twice at eight day intervals with iodized serum globulin. Metamorphosis occurred within twenty days. Control thyroidless animals injected with non-iodized globulin failed to transform.

3. Larval *Spelerpes* varying in length from 23 to 53 mm., were placed in strong solutions of tyrosine, dibromtyrosine, and diiodotyrosine. All animals of the iodotyrosine culture metamorphosed within twenty days. The larvae of the other cultures showed no indications of transformation.

The experiment shows clearly that iodine other than thyroid iodine is capable of inducing metamorphosis. The evidence indicates that metamorphosis is dependent upon a peculiar property of the iodine atom when combined in an unknown manner (not necessarily the combination occurring in the thyroid) because the iodine compounds effective in metamorphosing thyroidless amphibians have been shown to have no physiological effect upon thyroidless mammals.

39. *Experiments on metamorphosis.* (Lantern.) KARL E. MASON, Yale University.  
(Introduced by W. W. Swingle.)

1. Thyroidectomized and hypophysectomized *Rana sylvatica* tadpoles were reared in solutions of elemental iodine and fed starch iodide and spirogyra. Metamorphosis resulted, though the animals died before the tail was completely absorbed.

2. The thyroid glands of a large axolotl were extirpated, cut into ten pieces and each piece transplanted into a *Rana sylvatica* tadpole. Two pituitaryless, three thyroidless and five normal animals were engrafted. Three normal larvae were injected with the water in which the glands had been cut into pieces. The thirteen tadpoles metamorphosed within two weeks. Thus the thyroid glands of a single axolotl contained enough secretion to metamorphose thirteen thyroidless, pituitaryless and normal animals when transplanted or injected. The same glands left undisturbed within the animal's body were incapable of initiating metamorphosis. Axolotl neoteny is apparently due to failure of the thyroid glands to release the hormone.

3. A giant male *Rana catesbeiana* tadpole 160 mm. long and sexually mature was found to lack thyroid glands. They had evidently been present at one time because the animal had hind legs 22 mm. long. Neoteny due to spontaneous degeneration of the thyroid apparatus is uncommon in Anurans.

4. Efforts were made to metamorphose *Ammocoetes* larvae of *Petromyzon marinus* by rearing them in thyroxin solutions, injecting them with strong doses of this substance and lastly by engrafting them with large amounts of thyroid and pituitary tissue of adult frogs. The results were negative.

40. *The vascularity of the enamel-organ in the developing molar of the albino rat.*  
WILLIAM H. F. ADDISON and J. L. APPLETON, JR., University of Pennsylvania.  
(Introduced by C. E. McClung.)

In reviewing the previous work upon the vascularity of the enamel-organ, one notices the variability of the findings. In the albino rat, however, blood-vessels exist within the developing enamel-organ of the molars. They penetrate the external enamel epithelium and traverse the stellate reticulum to the stratum intermedium. They were not seen to penetrate the stratum intermedium and so probably do not come into direct contact with the ameloblasts. The entering blood vessels are always multiple. The time of vascularization of the enamel-organ is closely connected with the beginning of amelogenesis. Connective tissue in growth, following the route of the blood-vessels, plays a part in the ultimate disintegration of the stellate reticulum. A complete description appears in the current number of the *American Journal of Anatomy*.

41. *Experiments on limb posture in Amblystoma punctatum.* J. S. NICHOLAS,  
University of Pittsburgh School of Medicine. (Introduced by R. G. Harrison.)

Previous experiments have been reported which show that the girdle regulates the posture of the limb after the rotation of the limb bud. Subsequent experiments show that the parts which cause this regulation are located in a circle of tissue three-fourths of a somite wide surrounding the limb bud. The limb was rotated within this area, and the outer circle was rotated independently of the limb. These rotations have been carried out in both orthotopic and heterotopic locations. They

show that the limb posture is dependent upon the orientation of the surrounding tissue.

When a limb from one side is placed in the limb area of the opposite side, the outer circle of which has been inverted, there is no reversal of asymmetry of the limb. Reduplication is less frequent than in rotations of the whole limb disc of three and one-half somites.

The limb bud has been blocked by the transplantation of indifferent ectoderm. While this can be done, the distortion of the operative components during healing permits regeneration in a greater number of cases than is usual when the limb area is blocked in the usual location.

The portions of the girdle included in the circle of tissue around the limb bud are the supracoracoid, coracoid and procoracoid. When transplanted, these undergo a slight regulation in which they adjust themselves to the general conformation of the body wall. They maintain their transplanted location and orientation, causing the rotation of the limb bud during its development.

#### F. COMPARATIVE ANATOMY

44. *The origin and nature of the earliest neuromuscular connections in elasmobranch embryos.* H. V. NEAL, Tufts College, Mass.

A reinvestigation of the earliest stages in somatic nerve histogenesis in Elasmobranch embryos, and a critique of the conclusions of Dart and Shellshear ('22) and of Kerr ('19). The problem of the genesis of the first neuromuscular connections not insoluble, as asserted by Kerr, and not one to be settled by the selection of facts favorable to any hypothesis, however important, as has been done by Dart and Shellshear. The borax-carminic methods used by these authors are inadequate for the solution of problems of neurogenesis.

Neural tube and somite are not primarily in contact with each other, as stated by Dart and Shellshear, but are separated both in living specimens and in well-preserved material by a liquid-filled space free from plasmodesmatus connections. In formalin-fixed specimens plasmodesmata-like structures may result from plasmolysis. The absence of protoplasmic connection between tube and somite may not be correctly interpreted as a result of rupture in sectioned material.

The earliest protoplasmic neuromuscular connections in Elasmobranch embryos are secondary in formation, neurogenic in origin, and nervous in nature as shown by the presence of neurofibrillar substance. Contrary to the statement of Dart and Shellshear, there is no rythmical movement of Elasmobranch embryos of less than 4.5 mm. total length. Furthermore, there is no movement before nervous connection is established through the outgrowth of processes of medullary neuroblasts. Dart and Shellshear have presented no evidence adequate to establish their assumption of the myogenic origin of somatic motor neuroblasts, and no evidence whatever to prove the statement that the sympathetic is of mesenchymatous (mesodermal) derivation.

45. *An undescribed type of sense organ found in the larva of Botryllus.* CASWELL GRAVE, Washington University, and HELEN WOODBRIDGE, University of Maine.

One sense organ only is developed in the larva of Botryllus while two, an eye and a static organ, are typically present in the larvae of other compound ascidians. The



behavior of the Botryllus larva shows that definite and specific responses are made to stimuli by light and by gravity that are in no way different from the same responses made by ascidian larvae which have both an eye and a static organ. The morphology of the Botryllus sense organ reveals the fact that it is a combined eye and static organ. It consists of the following parts: a pigment cup attached by a slender stalk to the ganglionic wall of the sensory vesicle and a series of five nerve cells the cell bodies of which are located at the base of the sensory vesicle. From each cell body a robust fiber passes forward through the vesicle cavity to the mouth of the pigment cup. Within the cavity of the pigment cup each fiber becomes enlarged to form a sensory end organ which is intimately connected with a papilla in the floor of the pigment cup. The requirements for a simple static organ seem to be met by the fact that the sense organ as a whole hangs pendant within the vesicle by a slender stalk. The requirements for a simple direction eye seem to be met by the structural elements just enumerated, but it is noteworthy that lenses, which constitute a conspicuous element of the eye of other ascidian larvae, are wanting in the Botryllus eye.

46. *The morphogenesis of spines and spine-glands in the Siluridae.* H. D. REED, Cornell University.

The axillary dermal glands in catfishes comprise the whole thickness of the epidermis rather than the invagination and proliferation of the cells of the germinal layer. They possess at first no natural pore or lumen. These appear only with the active functioning of the gland.

In a few species there are glands of the same structural type associated with the pectoral and dorsal spines. These spine-glands at first appear to be contrary to all structural and developmental plans prevailing in those of the axillary region. The two kinds are seen to be in morphological harmony when the spine-glands are studied in the light of spine-morphology.

The spines are formed by modified soft rays in the form of conical caps applied one after another to the end. The soft rays of the fins are overlaid by epidermis abundantly provided with gland cells. The lepidotrichia contributing to the formation of the spines do not separate from the basement membrane thus carrying the epidermis to their final position in the spine. The spine glands are therefore, composed of a series of distinct superimposed glands which in the undisturbed estate are without pore or lumen.

47. *On specific characters in Teredo.* (Lantern.) THURLOW C. NELSON, Rutgers College.

A study of *Teredo* from various environments in Barnegat Bay, N. J., in 1921 and 1922, shows the presence of true *Teredo navalis*, L., together with *T. morsei*, Bartsch, and possibly other varieties. Sexually mature *Teredo navalis* obtained in 1921 from white cedar in the middle of Barnegat Bay, show typically 14 or 15 dental ridges, and other specific characters which closely agree with those of specimens from the coast of Holland. This Barnegat material has been identified by two of Europe's foremost authorities as *T. navalis*, L. *Teredo* in cedar and pine from tidal creek of Barnegat Bay in 1922 show from 25-30 dental ridges, and agree closely with description of *T. morsei*, Bartsch (*T. navalis*, var. *moresi*, Clapp). That the difference

between true *T. navalis* and var. *morsei* may be environmental rather than genetic and of specific value is suggested by the presence of intergraded forms. Photomicrographs are shown illustrating the characters of shells and of pallets in Holland and in Barnegat Bay Teredo.

48. *Intramuscular sensory endings of the small intestine, with a consideration of their central connections and probable function.* F. W. CARPENTER, Trinity College.

Intramuscular nerve endings of sensory type occur as minute telodendria in the muscular coat of the dog's small intestine. These arborizations (with the exception of a few in the subserous layer) have been found only in the longitudinal muscle embedded among and in direct contact with the muscle cells. The fibers with which they are connected have been traced into the myenteric plexus. Physiological and clinical evidence points to these arborizations as the pain terminals of the intestinal wall. The fibers which lead from them to the myenteric plexus are believed to be the peripheral processes of sensory neurones, the cell bodies of which are situated in the dorsal root ganglia of the tenth and eleventh thoracic nerves.

49. *Glochidial teeth and the mechanics of attachment.* LESLIE B. AREY, Northwestern University Medical School.

1. The flange of hookless and axe-head glochidia and the hook of the hooked forms are homologous structures. Both are specializations of the primitive valve rim with the undifferentiated remainder of which they are still continuous.

2. Externally the valves of glochidia are covered with a cuticular layer: internally the mantle epithelium is lined with a typical cuticula. The two become directly continuous at the shell's edge. At the region of the flange and hook the junction is on the inside surface and subterminal.

3. All the glochidial types bear teeth on their external surfaces. In the hookless and axe-head forms these teeth are extremely small ( $0.5-1.5\mu$ ) and numerous (several thousand). In the hooked glochidia they become relatively coarse spines along the median ridge of the hook; laterally they diminish progressively in size and may become as tiny as any in the other groups. As an extreme case (*Anodonta imbecillis*) these teeth extend along the rim and beyond the hinge line.

4. When attachment occurs, adduction causes the rim and flange (including its specialized hook) to cut the tissues until sufficient resistance turns them mechanically inward. Continued pressure flexes these parts at right angles to their original positions, and their external surfaces are opposed and parallel. During the inturning additional host tissue is engaged and locked by the teeth and spines. As a result the glochidium is more firmly attached by a broader zone of pinched and locked tissue.

50. *The gall-bladder in the cat,—its development, its functional periodicity, and its anatomical variation as recorded in twenty-five hundred specimens.* EDWARD A. BOYDEN, Harvard Medical School.

A pancreatic bladder was recently described by the author as developing from an accessory duodenal pancreas. The very existence of such a case seemed to argue against interpreting other types of pancreatic bladders as split portions of the cystic diverticulum. To test this hypothesis twenty-five hundred cats were examined. Three pancreatic bladders were found and over three-hundred multiple gall-bladders,

but no instance of subdivided bile-duct to bridge the gap. Conversely, fifteen aberrant pancreatic lobes were found which reached the gall-bladder and whose ducts reproduced, proximally, all essential conditions exhibited by pancreatic bladders of the usual type. Furthermore, the two pancreatic bladders which were sectioned differed histologically from the gall-bladder, and were pathological. A case similar to Professor Beckwith's (1918), in which an aberrant pancreatic diverticulum emptied into the neck of the gall-bladder, was also pathological, indicating that the connection in both cases was probably fistulous.

In addition to pancreatic and split gall-bladders a new aberrant type was found (twenty-nine cases) in which supernumerary bladders arose directly from the hepatic ducts. Only three instances occurred in which the gall bladder was absent.

Experimental feeding of cats shows that a meal of pure fat induces a cycle of changes in the gall-bladder: 1) a period of slow emptying; 2) a collapsed or resting period marked by a rearrangement of the mucosa; 3) a period of rapid filling and bile concentration. Pure protein or carbohydrate diet induces no resting stage. Mixed and superimposed diets produce various modifications of this cycle.

51. *The anatomy of an abnormal double monster (Duroc) pig.* (10 min.) GEORGE W. HUNTER and GEORGE M. HIGGINS, Knox College.

This pig, from a normal litter of six, was artificially delivered and died at birth. The monster has one head with normal mouth, nares and eyes; four external ears, two of which come out on the mid-dorsal line just back of an unexplored wart-like protuberance in the pineal position; eight legs, apparently normal. The bodies, one of which is much larger, are joined in the thoracic regions by their ventral surfaces.

The bodies have a common metacoel. Most of the viscera lie in the body of the larger animal, or in the common portion. The digestive tract is complete for the larger animal, although quite abnormal in some respects, but is incomplete in the smaller body. A relatively short intestine opens into a pouch in the intestine of the larger body.

There are two pleural cavities and two sets of lungs, placed dorsal side to dorsal side. Two trachea are found, the ventral one uniting with the single oesophagus near the larynx. Two hearts are present, dorsal surfaces opposed. Two dorsal aortas connect with each other and with the hearts. There are four jugulars, two precavae and two postcavae, although the venous system appears to be unconnected.

Normal kidneys and ovaries with normal ducts and blood connections are found in the larger body. Sex organs and kidneys are rudimentary in the smaller.

The dorsal vertebral columns appear to be connected in the thoracic regions. The glandular development, especially of the endocrine glands, is relatively great. The thymus, in particular is greatly developed.

## G. GENERAL PHYSIOLOGY

52. *The creeping of the larvae of the slug-moths (Cochliidiidae).* W. J. CROZIER, Rutgers College.

Caterpillars of four genera of the slug-moths, lacking pro-legs and having the thoracic legs so reduced as to be functionless in locomotion, creep by means of direct

neuromuscular waves confined to the adhesive ventral surface and similar in many respects to those pedal waves which are the means of progression in gasteropods. The nervous mechanism of coördination is different from that in mollusks, but the speed of the "pedal" wave is in quantitative agreement in the two cases. Reversal of creeping involves reversal of the locomotor wave. The speed of the reversed (retrograde) wave is greater than that of the direct, (by as much as one hundred per cent), although the former is relatively difficult to elicit. The temperature coefficient for the velocity of the "pedal" wave is about 2.3.

53. *Photoreceptors of Lumbricus terrestris.* WALTER N. HESS, DePauw University.

It is generally known that earthworms are sensitive to light, but little is known concerning the distribution and nature of the photoreceptors.

By the use of ordinary illumination it is possible to demonstrate that worms of this species are most sensitive to light in their anterior regions, somewhat less in the posterior, and least of all, in the middle portions of their bodies.

By means of a strong pin-hole light, the prostomium and the three anterior segments were found to be most sensitive, of which the prostomium appeared to be slightly more sensitive than the rest. Although all segments were found to be photosensitive, each segment, with the possible exception of the first three and the last one or two, was most sensitive in the anterior portion of the dorso-lateral region. No reactions were obtained by illuminating the mid-dorsal areas except those of the twelve anterior and the three posterior segments. The worms did not react to illumination on the mid-ventral surface except on the three anterior and the last segments.

Some of these conclusions were confirmed by tests which were made, following the removal of certain anterior segments and certain parts of the nervous system.

A comparison of the histological structure of the more sensitive with the less sensitive regions, revealed the fact that a rather peculiar type of sense-cell is more abundant in the former than in the latter areas. This indicates that these sense-cells are photoreceptors.

54. *Hibernation in Orthoptera. I—Physiological changes during hibernation in certain Orthoptera.* JOSEPH HALL BODINE, University of Pennsylvania.

Some physiological changes during hibernation in grasshoppers, such as changes in the water content, the length of life, the rate of growth, the rate of metabolism, etc., are pointed out. It is shown that *Chortophaga viridifasciata* in the vicinity of Philadelphia, hibernates rhythmically at a definite stage in its life cycle and that during hibernation the animals take in food. The animal's ability to withstand cold seems to be related to its water content and the various changes in the percentage of water make it a rather unique organism. During hibernation animals do not grow nor gain in body weight. Hibernating animals, brought and left at room temperature (20° C.) after a short interval during which water content readjustments take place, grow in a normal manner. Rate of recovery from hibernation is quickest in animals brought indoors latest in the hibernation period. Differences in rate of recovery from hibernation, rate of growth, rate of CO<sub>2</sub> output, etc., for males and females are pointed out. Rates of CO<sub>2</sub> output for hibernating animals at 0° to 8° C. are noted,—being eight to ten times lower than at room temperature (20° C.).

Rates of  $\text{CO}_2$  output in hibernating animals at room temperature remain higher than in growing animals, suggesting that the animals remain young throughout the period of hibernation. *C. australior*, a species in which hibernation had not occurred, when subjected to hibernating conditions reacts similarly to *C. viridifasciata*. It thus seems possible to induce hibernation in closely related species of animals.

55. *Some reactions of Alligator mississippiensis.* ALBERT M. REESE, West Virginia University.

*Temperature.* When given a choice of pans containing fresh water at  $10^\circ \text{C}$ .,  $25^\circ \text{C}$ . and  $40^\circ \text{C}$ ., approximately 16% of the observations showed the animals in the coldest water, 60% in the medium temperature, and 24% in the warmest water.

*Sea-water.* When given a choice between pans of fresh and sea-water the animals gave about 54% to 46% in favor of the fresh water, indicating practically no preference of one medium over the other.

*Light.* When given the choice between the shaded end of a black painted box and the end illumined from above by a tungsten lamp the animals showed a preference for the dark end in the proportion of 62% to 38%.

*Chemical.* As might be expected, the tough hide of the animals seems quite unaffected by ordinary chemicals, 5% NaOH or 20% acetic acid producing no reaction. Small wads of cheese-cloth moistened with volatile and odoriferous liquids produced varying responses when suspended near the nostrils. For example the responses to chloroform and ammonia were very marked, often violent, while to concentrated hydrochloric acid, so irritating to the human olfactory membranes, the response was often much less marked. To some of the strong smelling but non-irritating oils no response was noticed.

*Food.* The animals accepted every kind of animal food that was offered, live animals being captured partly by sight, partly by the disturbance of the water and partly by chance.

56. *Quantitative observations on the digestive power in Amphibian larvae during metamorphosis.* ALBERT KUNTZ, St. Louis University School of Medicine.

Metamorphosis in Amphibia involves a coincident period of fasting during which the secretion of digestive enzymes is markedly retarded. Quantitative data are presented which indicate almost complete suspension both of peptic and amylolytic activity in *Rana pipiens* and *Ambystoma tigrinum* during metamorphosis and rapid acceleration of these activities as metamorphosis is completed and feeding is resumed.

57. *The effect of light on various marine invertebrates.* A. G. HUNTSMAN, Atlantic Biological Station.

Several days' exposure to direct sunlight is found to be fatal to a number of species of marine invertebrates with which experiments have been made. These consist of Crustaceans (e.g. *Calanus* and other Copepods, a Caprellid and a Gammarid among Amphipods, the Schizopod, *Meganyctiphanes*, and the larva of the lobster), Ascidians (the genera *Molgula*, *Boltienia* and *Tethyum*), and a Coelenterate (*Alcyonium* or *Gersemia*). In the experiments controls were used, for which the conditions were kept the same as for the individuals in the experiments proper, except in regard to light. The thesis is advanced that the direct action of light determines the upper bathymetric limits of many marine animals.

58. *The axial gradients in Corymorpha palma.* C. M. CHILD, University of Chicago.

The existence of a physiological gradient in the main axis was demonstrated by the susceptibility method with KNC and other agents, by reduction of  $\text{KMnO}_4$ , by vital staining with neutral red and methylene blue, by decoloration of methylene blue, and by comparative colorimetric estimation of  $\text{CO}_2$  production. By all these methods except the last a gradient was also demonstrated in each tentacle, each gonophore and each frustule. The apico-basal gradient was also shown to be present in the planula and in early stages of hydroid development and it was found that a new gradient appears in the development of every new axis in the reconstitution of pieces. In short pieces it was possible to determine new gradients and so new axes by differential exposure to environmental conditions.

Estimation of  $\text{CO}_2$  was made with phenol red as indicator. Hydranths and stems, apical and basal halves, and apical, middle and basal thirds of the naked region of the stem were compared and all experiments showed a decrease in rate of  $\text{CO}_2$  production in the basal direction. In extreme basal regions growth of frustules increases the rate somewhat.

Small sexually immature animals show much higher susceptibility, reduce permanganate more rapidly, stain more rapidly with neutral red and methylene blue, decolorize methylene blue more rapidly and produce  $\text{CO}_2$  more rapidly per unit of weight than large, sexually mature animals.

59. *Oxygen consumption with respect to level, size, and regeneration and electrical polarity in Corymorpha palma.* (By title.) L. H. HYMAN, University of Chicago.

Tests were made of the rate of oxygen consumption per unit weight of different levels of the stem of *Corymorpha*. In all stems used the apical cut is made below the neck region of the hydranth and the basal cut above or at the upper rim of the perisarc. Apical halves of such stems consume more oxygen than basal halves (eight experiments, all positive, average difference, 22 per cent). Apical thirds consume more oxygen than middle thirds (six experiments, all positive, average difference, 40 per cent); and middle thirds more oxygen than basal thirds (six experiments, all positive, average difference, 20 per cent).

The rate of oxygen consumption per unit weight is greater the smaller the individuals; in four experiments, 10–15 mm. specimens consumed 130–300 per cent more oxygen than 40–50 mm. specimens. The difference applies to both stems and hydranths.

Pieces after regeneration have a markedly greater rate of oxygen consumption than the same pieces before regeneration (five experiments, all positive, average increase, 600 per cent).

Hydranths are electronegative (in the galvanometer) to stems and distal levels of stems negative to proximal levels down to the zone of stolons (frustules). This zone is decidedly negative to the stem just distal to it.

60. *The manner of copulation in triclad Planarians.* R. A. BUDINGTON, Oberlin College.

The behavior of triclad flatworms during copulation, if indeed it has been accurately observed, seems not to have been described in monographs on behavior in this group, or to be incorporated in current texts.

Unlike the method in *Lumbricus* (also hermaphrodite), the two copulants, in the instance of *Planaria maculata*, are oriented in the same direction, side by side, their anterior ends placed flatly on the substratum, their posterior halves so twisted that the ventral surfaces (and consequently genital atrial pores) are closely flattened, the one against the other. The copulatory organ of each worm is protruded through the atrial pore for about 1 mm., at the same time in both copulants, so that exchange of spermatozoa is mutual and simultaneous. The spermatozoa seem to be taken at once and practically wholly into the uterus, sections of ex-copulants showing the uterus and its duct to contain large masses of sperm. Distinct spermatophores, while described for certain other species of *Planaria*, are not apparently formed by *Planaria maculata*.

61. *The olfactory sense of the cabbage butterfly, Pieris rapae Linn.* DWIGHT E. MINNICH, University of Minnesota.

The cabbage butterfly, *Pieris rapae Linn.*, extends its proboscis upon appropriate olfactory stimulation, and this response may be used in locating the olfactory organs. Butterflies collected in the field generally become 100 per cent responsive or nearly so after twenty-four to thirty hours of total inanition. If such animals are subjected to operations eliminating the antennae from possible olfactory function, through coating with vaseline or paraffin-vaseline mixture, or through amputation, the response to olfactory stimulation is decreased by 50 to 80 per cent. If only one antenna is eliminated, the decrease is but one-third to one-twelfth this amount. Strictly comparable data may be obtained by removing one antenna, then the remaining antenna, and making a set of trials after each operation. A group of twenty-two animals, twelve males and ten females, was tested in this manner. Before any operation they gave 100 per cent response. After the removal of one antenna, they gave 89 per cent response. After the removal of both antennae, they gave 47 per cent response.

It is thus clear that the elimination of one antenna alone produces a relatively small decrease in response, while the elimination of both antennae effects a great reduction in the same. Even the elimination of both antennae, however, fails to abolish the response. Two conclusions may be drawn from these facts: first, the antennae of *Pieris* bear important olfactory organs; second, the olfactory organs are not limited to the antennae.

62. *The stimulating efficiency of intermittent light in the drone-fly, Eristalis tenax.* WILLIAM L. DOLLEY, JR., Randolph-Macon College.

The stimulating efficiency of intermittent light in *Eristalis* may be greater than, equal to, or less than that of continuous light, depending upon the flash-frequency. Five flies were tested in intermittent light of an average illumination of 92 meter-candles and of various flash-frequencies. At flash-frequencies of 14, 20, 25, and 33 per second the stimulating effect of intermittent light was greater than that of continuous light of equal illumination; at flash-frequencies of  $2\frac{1}{2}$  and 5 per second it was less; and at flash-frequencies of 10, 40, and 50 per second it was about equal to that of continuous light.

The flash-frequency of maximum stimulating effect varies with the illumination. Each of five insects was tested in intermittent light of 10 and 570 meter-candles.

In the former illumination the flash-frequency of maximum stimulating efficiency was about 14 per second, while in the latter it was approximately 33 per second. Consequently, the stimulating efficiency of intermittent light in *Eristalis* depends upon the flash-frequency and the luminous intensity.

63. *The chemical sense of Palaemonetes vulgaris* (Say). MANTON COPELAND, Bowdoin College.

Experiments show that *Palaemonetes vulgaris* finds fish meat concealed in sand, or in a tube covered with cheesecloth and suspended two centimeters from the bottom of an aquarium. Reactions to a baited tube are much more frequent than to one placed nearby similar in appearance but containing no food. The prawn is successful also in finding meat when either the first or the long filaments of the second antennae are removed. When, however, both pairs of organs are excised the location of the food is less frequently or precisely determined, although the behavior of the animal shows unquestionably that it is being sensed. It becomes evident, therefore, that neither the first nor the second antennae are to be regarded as specialized olfactory organs, although both may play an important part in food discovery. The sense of sight is a minor factor in these reactions.

Numerous tests make clear that *Palaemonetes* is sensitive to chemical stimuli over the greater part of the surface of the body, and that it may exhibit remarkable differential response to localized stimulation.

The animal also senses food when the stimulating material is more dilute than in the cases cited, and gives indication of directive response.

The reactions recorded appear to depend on a diffused integumentary sense which, in certain respects at least, resembles more closely the olfactory sense of higher animals than either the gustatory or common chemical sense.

64. *Sterility of animals under changed condition.* (By title.) CARL HARTMAN, University of Texas.

Many animals are known not to mate in captivity; others are sterile in spite of matings. Sterility in the opossum due to atresia of the ovarian follicles has been encountered for some years, the proportion of cases increasing with the length of captivity. Normally the female opossum ovulates in absence of the male; conversely, the presence of the male does not (as in the rabbit) prevent atresia of the ripe or nearly ripe follicles.

In experimenting upon 65 females from March to May, 1922, an attempt was made to study the possible rôle of vitamins in the present instance (cf. Evans and Bishop, 1922). All animals received bread and skim milk; and certain groups received an addition of liberal quantities of whole milk, cod-liver oil, yeast or canned tomatoes, or combinations of them. Normal ovulation took place in certain individuals without regard to quantity or kinds of vitamin; in others super-abundance of fat soluble A, in combination with B and C, failed utterly to prevent or cure the atresia.

Finally six animals, all of which had been in dioestrus (epithelial cells in vaginal smear) for months, were allowed the free run of a room 8 x 20 feet. Four of these promptly ovulated. Though the number is small the result is in line with the observations of others that exercise favors fecundity.

As the animals were otherwise in apparently excellent health, the ovary appears to be the most delicate indicator of bodily dysfunction (cf. Stieve, 1918; Evans and Bishop, 1922).



65. *The attachment of oyster larvae.* (Lantern.) THURLOW C. NELSON, Rutgers College.

Mature oyster larvae were observed in the act of setting on a glass plate which had been suspended in a tidal creek containing oyster larvae at the setting stage. The larvae moved slowly about on the foot, describing circles of ever decreasing diameter, coming to rest after about 15 minutes with the ventral border of the left valve in contact with the glass. The foot, held in the median position, kept the shell in place while attachment was effected by bringing the secreting edge of the mantle border in contact with the glass. The circling of the larvae prior to attachment results in a strikingly even distribution of the attached spat.

66. *The circus movements of Limulus.* WILLIAM H. COLE, Lake Forest College.

Symmetric and asymmetric stimulations of Limuli (20-60 mm. diam.) have revealed positive phototropism and positive circus movements. The circle diameter and the light intensity are inversely proportional. Three intensities of non-directive, diffuse illumination were used, being approximately 8000, 2000, and 900 cm. In some animals the lateral and median eyes on one side were removed, and in others these eyes were covered by asphaltum. Under intensity I the mean turning per centimeter was  $6^{\circ}.73$ ; under intensity II,  $5^{\circ}.23$ ; under intensity III,  $4^{\circ}.78$ . These figures came from 133 experiments on 38 animals, selected because of their regular reactions. About 25 per cent of normal animals were irregular in their reactions, some being indifferent to light, others showing a mixture of positive and negative reactions, as might be supposed, since in many animals primitive tropisms are frequently modified or even obliterated by other reactions occurring simultaneously.

The inverse relation between light intensity and diameter of the circles in Limulus contributes further evidence to Loeb's heliotropism theory formulated in 1888. Under non-directive diffuse illumination the strength of the stimulus on the asymmetric photo-receptor remains practically constant during a single revolution, and all parts of the retina are equally stimulated, since the rays of light enter at all angles with the same intensity. This fact would vitiate any hypothesis based upon localized retinal stimulation in animals with eyes. The few exceptions to the tropism theory so far reported are undoubtedly due to the masking of the primitive tropistic reaction by other more complex reactions.

67. *Effect of thyroid feeding on the color and form of the feathers of fowls.* BENJAMIN HORNING and HARRY BEAL TORREY, University of Oregon.

Brown Leghorn chicks from the age of two weeks were given dried thyroid in daily doses proportionate to their size. In these birds the assumption of adult plumage, both as to form and color was delayed. The coloring of the birds, at the present time seven months old, still departs in certain definite respects from the normal. The general color is darker, owing to a marked increase in black pigment in hackles, shoulder, back and saddle feathers. The brilliant red characteristic of the normal hackles, back and saddle feathers is poorly developed or lacking, its place being taken in part by yellow. The hackles and saddle feathers show a sort of spotting near their ends, due to irregularities of the black central stripe that interrupt the continuity of the highly colored border.

The color change is associated typically with a structural change, the increase

in width of the dark central stripe being accompanied by a corresponding increase in the number and distribution of the barbules on the barbs.

68. *The physiological response of Paramecium to thyroxin.* MATTHEW C. RIDDLE and HARRY BEAL TORREY, University of Oregon.

The physiological response of *Paramecium* to the presence of thyroxin crystals in the culture medium is prompt and definite. The delay or lag which Kendall observes in vertebrates is absent.

The effect of the thyroxin is manifested especially in a slight decrease in the rate of division and a large increase in excretory activity. The average daily division rate calculated from observations on eighteen experimental cultures, under observation for periods ranging from seven to seventy-one days, was 0.8205 after thyroxin, 0.8587 in the controls. These figures represent a decrease of 4.54 per cent in division rate in the thyroxin cultures. This decrease is too small to be of little more than negative significance. It is in sharp contrast, however, with the large increase in division rate observed by Shumway in *Paramecium* fed with thyroid gland. The contrast suggests that factors other than thyroxin may be responsible for Shumway's results.

The acceleration of the excretory activity of *Paramecium* by thyroxin is indicated by an increased rate of pulsation of the contractile vacuoles, an increase in the number of accessory canals, and a great decrease in the number of excretory crystals in the endoplasm. These observations are in accord with those of Shumway and other investigators who have analyzed the response of *Paramecium* to thyroid gland.

Thyroxin appears to promote katabolic rather than anabolic processes, a view in accord with the idea advanced by Biedl that the active agent in the thyroid secretion is a dissimilatory hormone.

69. *Excretion and death among amebas.* A. A. SCHAEFFER, University of Tennessee.

The length of time that undigested remains of food are kept in the different species of amebas varies very greatly. Some species excrete undigestible materials within a half-hour or less, after feeding, and are consequently frequently seen without any recognizable food objects in them. Other species retain much if not all of the indigestible material indefinitely. Reproduction by division prevents the excessive accumulation of these materials in the body. The crystals, a by-product of metabolism, are also not excreted nor are they destroyed within the ameba. The excessive accumulation of these crystals is also prevented by division. But when the division rate slows down or when division ceases altogether, the inert materials accumulate until the ameba finally dies. These and other observations indicate that cells which excrete imperfectly will die if they do not divide.

70. *The physiological action of excretory products.* EDWARD F. ADOLPH, University of Pittsburgh.

It is usually assumed by biologists that the end-products of metabolism are toxic to the cells producing them. This is known to be true in metazoan cells for carbon dioxide and for acids in general, but not in green plant cells for oxygen. Are the nitrogenous excretory substances detrimental to animal tissues? The flatworm *Phagocata* lived for 24 hours or more in a 0.31 M urea solution, while Sucrose solu-

tions and Ringer's solution above 0.23 M are toxic, as are sodium chloride and other salt solutions of equivalent osmotic pressure. Gradual acclimatization led to survival in slightly higher concentrations. Paramecium lived for one hour or more in urea, sucrose, and salt solutions of 90 per cent of the above concentrations respectively. Uric acid and the most soluble neutral urates, monolithium and monopotassium form saturated solutions of insufficient concentration to have any physiological effect. Hearts of the newt *Diemyscylus* were immersed in Ringer's solution containing varying quantities of urea up to 0.28 M without measurable physiological detriment. A human subject ingested 45 grams of urea (0.65 grams per kilo) at a single dose without symptoms. The evidence thus accumulated fails to indicate that the urologist's uremia is due to urea, and that gout and other diseases are at all complicated by the presence of excessive amounts of urates in the organism. Either waste-products are not deleterious through their retarding of metabolism, or urea is not an excretory substance in Phagocata and Paramecium. Apparently all tissues can withstand any concentration of urea which is physically compatible, and the tissues of selachian fishes are not alone in their tolerance of this substance.

71. *Eyes in Volvox and their function.* S. O. MAST, Johns Hopkins University.

The eye-spots in *Volvox* consist of a brownish cup-shaped structure and a hyaline lens-shaped body. There is one in each zooid, some colonies containing as high as 22000. Those at the anterior are much larger than those at the posterior end of the colonies, varying from  $3\mu$  to less than  $1\mu$  in diameter. They are situated at the postero-lateral surface of the zooids and face postero-laterally.

The cup is transparent for the longer but opaque for the shorter waves. If direct sunlight is thrown into it the former (yellow, red) penetrate the wall and come to a focus near the convex surface while the latter (green, blue) are reflected by the inner surface of the cup and come to a focus between it and the lens. The region of maximum stimulating efficiency in the spectrum is in the green near the blue. The photosensitive substance is consequently probably located at the blue-green focal point, between the lens and the inner surface of the cup.

When the colonies are laterally illuminated, rotation on the long axis results in alternate decrease and increase in the intensity of the blue-green in the cup. The former causes an increase, the latter a decrease in the backward stroke of the flagella if the colonies are positive, and the opposite if they are negative. These responses are dependent upon time-rate of change in intensity. They are of the all-or-none or trigger type.

The eye-spots in *Volvox* function as direction eyes.

72. *The growth of marine organisms on submerged metals.* G. H. PARKER, Harvard University.

Pieces of Al, Zn, Fe, Sn, Pb, and Cu were submerged in the sea and the growth of animals upon these metals was recorded. A general growth occurred on Al, Fe, Sn, and Pb, a very slight one on Zn, and none on Cu. Apparently marine animals will grow on any heavy metal provided that metal does not liberate ions or soluble compounds. The ions and soluble compounds of the heavy metals are usually extremely poisonous and where they are liberated freely from a metallic surface that surface is protected against organic growth. Such seems to be the case with Zn and

Cu. With Al, Fe, Sn, and Pb the products of marine corrosion are essentially insoluble and hence organisms grow on these metals in the sea. By coupling Cu with members higher in the electromotive series, this metal can be rendered chemically inactive in seawater and under such circumstances animals will grow freely upon it. Zn in this respect is much less easily controlled, for it lies high in the electromotive series and consequently it is not open to the electromotive protection that Cu is. Its compounds, moreover, are relatively freely soluble and thus become very effective in checking the growth of animals.

73. *Relative effects on ciliary activity of anion and undissociated molecule of organic acids.* J. M. D. OLMSTED and J. W. MACARTHUR, University of Toronto.

The question has been raised as to the cause of the greater effectiveness of organic acids over inorganic acids of the same pH in inducing changes in certain physiological processes, *e.g.* the stopping of ciliary action. Both anion and undissociated molecule have been held responsible. To differentiate the effect of the anion from that of the molecule we have used combinations of acids and non-injurious salts, such as HCl + NaCl, HAcet + NaCl, HAcet + NaAcet, HCl + NaAcet, etc. The mixtures were in every case essentially isotonic throughout the entire pH range, thus eliminating the important osmotic factor. By taking into consideration the rôle of ionization in these combinations, it is possible to determine accurately the relative effects produced by the anion and the undissociated molecule. In the case of acetic acid the effect is evidently due preponderantly to the undissociated molecule.

74. *On the nervous organization of Limax.* W. J. CROZIER, Rutgers College.

Reciprocal systems of muscles concerned with extension and retraction of the head and the mantle of *Limax maximus* have their normal actions completely reversed following suitable injection with strychnine. The sensitivity of the animal is so increased that gentle breathing upon it may suffice to elicit vigorous retraction of head and violent forward extension of mantle. Strychnine is in this respect unique among alkaloids tested. It does not influence local responses of the body wall. Its chief effect is probably upon the central ganglia, as in *Chromodoris*. Other neurophil substances likewise produce in *Limax* effects of highly specific quality; such that a number of neuromuscular elements in the motor system of the slug may be analytically separated. Thus nicotine, in *Limax* at the male phase, leads to complete extension of the intromittent organ. The results are useful toward an account of the nervous organization of gasteropods, and also for their bearing upon the specificity of nervous differentiation.

75. *Concerning laws of locomotion in gasteropods.* W. J. CROZIER, Rutgers College.

An attempt to discover correlations among physical features of the creeping of *Limax*. Vlés has proposed equations connecting frequency of pedal waves, velocity of creeping, and the power to carry the body of the animal vertically. These expressions fail when observations from any single individual are studied, but they have a certain average validity when results from numbers of individuals are massed; their physical meaning is therefore obscure. When *Limax* creeps with pedal waves of a given conformation, the velocity of progression is proportional to wave frequency. But the efficiency of the single wave may be changed independently of the frequency.

76. *Effect of thyroid feeding on the moulting of fowls.* BENJAMIN HORNING and HARRY BEAL TORREY, University of Oregon.

Brown Leghorn chicks were fed dried thyroid in doses progressively larger with increase of body weight, beginning with one-half grain two weeks after hatching.

One result was a striking alteration of the normal course of moulting. Customarily the first and second sets of rectrices are quite separate and distinct. In the thyroid fed birds, however, they are continuous. The feathers of the first set develop each with an abnormally short vane, leaving the shaft naked for a longer distance than usual. Each feather is pushed out by what appears to be a feather of the second set, with which it is continuous. After remaining attached to the latter for a few days it breaks off, leaving the tip of the new feather frayed and ragged, but otherwise in form typical of the feathers of the normal second set.

Similar interference with the normal moult has been observed in other regions, notably in the wing coverts. The appearance of characteristically male plumage (e.g., hackles and saddle feathers) is retarded several weeks in thyroid fed males. There is some evidence for the view that this retardation is not so pronounced in thyroid fed capons.

77. *The reactions of larvae of *Vanessa antiopa* Linn. to sound.* DWIGHT E. MINNICH, University of Minnesota.

The full grown larvae of the mourning-cloak butterfly, *Vanessa antiopa* Linn., respond to certain sounds by contracting certain of the longitudinal muscles in such a manner that the anterior third of the body is thrown dorsally or dorso-laterally. Varying intensities of response may be elicited; from extremely violent jerks, produced by intense sounds, to barely visible retractions of the head, effected by stimuli near the threshold.

Trials with tones show a very definite upper threshold with respect to pitch. This threshold is between d'', 576 v.s., and b', 480 v.s. Above this threshold even very intense tones seem to elicit responses from only occasional individuals; as a rule. Below this threshold, however, the caterpillars react regularly and vigorously down to and including tones of 32 v.s.

Mechanical vibration of the substrate does not effect the response in question, although a sudden sharp air current will. In contrast to a sudden jet of air, a continuous air current, even though gentle, will completely inhibit the response to sound.

The spines may be removed from the caterpillar without abolishing the response to sound. An attempt was made to eliminate the body hairs also, by means of singeing. The animals did not respond after this operation, but the violence of the procedure resulted in the death of all the animals except one within a few hours after the operation. Consequently, no conclusion may be drawn concerning the hairs until further work is done.

78. *The process of photic orientation in *Volvox*.* S. O. MAST, Johns Hopkins University.

The flagella in *Volvox* beat diagonally, resulting in locomotion and rotation on the long axis. If the illumination is suddenly reduced the flagella beat straight back for 1 to 3 seconds resulting in momentary cessation in rotation and increase in the rate of locomotion. If the illumination is increased the opposite occurs, locomotion

ceases momentarily and the rate of rotation increases. These responses do not occur if the illumination is gradually changed. They are consequently dependent upon the time-rate of change in luminous intensity.

In positive colonies, laterally illuminated, the flagella on the shaded side beat backward more effectively than those on the illuminated side resulting in turning toward the light (in negative colonies the reverse obtains).

In the zooids on the illuminated side of the colonies the light shines into the eye-cups and the photosensitive tissue is illuminated, in those on the shaded side this tissue is shaded by the wall of the cup, the amount of light it receives is consequently greatly reduced when, owing to rotation, the zooids proceed from the illuminated to the shaded side of the colonies, and greatly increased when they return. In positive colonies this results in increase in the backward stroke of the flagella on the former side and decrease on the latter side, and turning toward the light.

After the colonies are oriented and face the light directly the photosensitive tissue is no longer subjected to changes of intensity and they consequently proceed directly toward it.

79. *On certain determining factors in regeneration.* J. WILLIAM BUCHANAN, Yale University. (Introduced by L. L. Woodruff.)

Concentration of chloretone that have been shown to be effective in controlling head frequency in pieces of *Planaria dorotocephala* when applied immediately after section during the period of stimulation, are also effective in controlling head frequency when applied after the stimulation of section has disappeared.

Concentrations of ether that are effective in controlling head frequency when applied immediately after section are *ineffective* in producing increases in head frequency when applied after the stimulation of section has disappeared; only decreases in head frequency appear.

Measurements show that chloretone in the concentration and duration of exposure employed is an effective general protoplasmic depressant; and that ether in the concentration and duration of exposure employed is not effective in producing general protoplasmic depression.

The facts indicate that the metabolically more active tissues of the developing anterior end recover more rapidly from the depressing effect of chloretone than do the less active tissues of the piece; this recovery while the remainder of the piece is still depressed tends to increase head frequency; that long continued exposure to the depressing action of chloretone so affects the young, highly susceptible tissues of the developing anterior end that recovery is incomplete; and tends to decrease head frequency; that no increases in head frequency appear after exposure to ether because this substance is not effective in depressing the metabolic activity of the old tissues of the piece; apparently only the highly susceptible tissues of the developing anterior end are appreciably affected; hence only decreases in head frequency appear.

80. *Weight changes and oxygen consumption during long exposure to dilute anesthetics.*

J. WILLIAM BUCHANAN. (Introduced by L. L. Woodruff.)

When individuals or pieces of *Planaria dorotocephala* are subjected continuously to mol.1/10 alcohol the oxygen consumption increases much more rapidly than that of control animals. With animals of moderate size the oxygen consumption in alcohol

at the end of six weeks is seven hundred per cent that of control animals. The susceptibility of the alcohol animals to lethal solutions of KCN is also markedly greater than that of control animals. The weight of the alcohol animals decreases only slightly more rapidly than that of the controls. The data suggest that the alcohol is oxidized with increasing rapidity by some intrinsic oxidative mechanism of the organism.

Subjecting animals to mol.1/3000 chloroform for two weeks or less induces slight increases in oxygen consumption over the control animals and greater decreases in weight. Subjecting the animals to mol.1/825 chloral hydrate induces greater increases in oxygen consumption than in the case of chloroform but not so great as in alcohol. The weight decreases more rapidly than that of control animals. Incomplete work indicates that long exposure to mol.1/1000 chloretone also induces an increase in oxygen consumption above that of controls and also that their weight decreases more rapidly. Similar experiments with mol.1/300 ether failed to show any effect of this concentration on oxygen consumption and decrease in weight. The data suggest that the normal respiratory metabolism is stimulated by these agents, and that alcohol and perhaps also chloral hydrate are oxidized by the organism.

81. *Amoeboid movement and coalescence of dissociated sponge cells.* PAUL S. GALTISOFF, U. S. Bureau of Fisheries. (Introduced by W. C. Allee.)

The examination of various sponges (*Microciona*, *Cliona*, *Reniera*, *Halichondria*, *Grantia*), made by the writer, shows that the coalescence of dissociated sponge cells occurs as a result of the aimless amoeboid movement of the archaeocytes moving in different directions and coalescing with cells which happen to lie in their route. The outer protoplasmic layer of archaeocytes is sticky; this allows the cells to adhere to one another and to various objects which they meet in their route.

The coalescence of cells of two different species never occurs apparently because the physical properties of protoplasm of the various species are different.

The adhesiveness of protoplasm of *Microciona* cells may be increased by raising the alkalinity of water. In this case, when *Microciona* and *Cliona* cells are mixed together, the *Microciona* aggregates are surrounded by *Cliona* aggregates, the latter forming a ring closely attached to the former.

The presence of cells of another species mutually suppresses their amoeboid movement. Consequently the aggregates formed in a compound suspension are smaller and correspondingly more numerous.

The phenomenon of coalescence of dissociated sponge cells has a great resemblance to the agglutination of motile blood cells of Arthropoda. In both cases no chemotropic or "cytotropic" stimulus can be discovered and no such hypothesis is required to explain the results.

82. *Reversal of vertebrate heart beat.* JAMES NELSON GOWANLOCH, Wabash College. (Introduced by B. H. Grave.)

Striking and significant modifications of heart form and function can be produced in a teleost (*Macropodus viridi-auratus* Lacepede) by the action of various chemical and physical agents during early development. A differential inhibition or obliteration of the heart is possible, beginning at the sinus end and a graded series of forms

ranging from normality to complete absence of heart has been produced. Physiologically certain of the differentially inhibited, tubular hearts closely approximate the ascidian condition, groups of beats of conus origin alternating with groups of sinus origin. In other cases a continuous conus to sinus beat is established, constituting a complete reversal of the normal direction of beat. These modifications of form and function can be experimentally controlled to a very considerable degree and it seems entirely clear that the basis of such control of form and beat is an effect upon a graded differential in metabolic activity, and that, whatever may be the nervous or muscular mechanisms of integration in the definitive heart form, its primary integration is due to no such mechanisms, but to a fundamental gradient in metabolism with low end at the conus and high end at the sinus. The results of this work are in complete agreement with, and constitute further evidence in support of the axial gradient conception.

83. *Observations on the ciliary action of Scyphidia.* RUTH JANE BALL, University of Vermont. (Introduced by H. F. Perkins.)

There are four distinct groups of cilia in Scyphidia: a single row of fine discal cilia; two groups of setaceous appendages at opposite points on the disc; two long curved "vestibular setae" attached to the lower side of the gullet; and the fine cilia of the gullet. The discal cilia bring particles to the peristome border. Their movement is not constant and is controlled by the two groups of setaceous appendages that move by a characteristic "twitching." The fine cilia of the gullet are constantly in motion. Their direction of beat is often reversed. The "vestibular setae" aid in pushing away solid contents of extruded food vacuoles and in capturing large particles. A detailed study of each group has been made. Illustrations and a discussion of each group, with especial reference to ingestion and rejection of food and foreign particles.

84. *The effect of temperature upon the rate of retinal pigment migration in crustaceans.* RUDOLF BENNITT. (Introduced by G. H. Parker.)

Dark-adapted Gammarus were subjected to different constant temperatures, the duration of the distal pigment migration induced by light being found by killing and sectioning individuals at successive stages during exposure to light. The duration of the proximal migration induced by darkness was similarly determined in light-adapted Gammarus. In both migrations, the curve of temperature against rate follows closely that of Vant Hoff's Law of the relation of temperature to rate of chemical processes. Retinal pigment migration is hence a biochemical, rather than a physical, process, and is based upon protoplasmic streaming within the retinal cells.

85. *The feeding reactions of the ciliate, Dileptus gigas, with special reference to the function of the trichocysts.* J. PAUL VISSCHER, Johns Hopkins University. (Introduced by S. O. Mast.)

Dileptus gigas normally feeds on living organisms. It discriminates between living organisms and inanimate substances, ingesting the former in large amounts while the latter are only sparingly ingested. It selects from among the different kinds of organisms, eating some with great readiness while others are rarely ingested. It captures its prey by means of trichocysts which are embedded in a groove on the



oral surface of the proboscis, forming a median band extending from the tip posteriorly to the mouth. These trichocysts either paralyze the prey or cause cytolysis of its protoplasm. They are probably of a liquid nature, highly toxic, with specific cytolytic properties. They are sac-like structures whose contents remain liquid after they are discharged. For this reason the term toxicyst is suggested instead of the term trichocyst.

Selection of food in *Dileptus* is dependant on two factors; first, the physiological state of the organism, which determines whether a substance shall be ingested in large or small amounts; and second, the chemical properties of its trichocysts, which in relation to the properties of the protoplasm of its prey, determine in large measure whether a living organism can or can not be successfully captured. Specialized structures as for example, the trichocysts of *Paramecium* and the lorica of *Euplotes*, serve as protection against the attacks of *Dileptus*. The power to select food in *Dileptus* is dependent on physical and chemical factors.

#### H. ECOLOGY AND ZOOGEOGRAPHY

88. *Quantitative aspects of association and of seasonal succession in an artificial environment.* W. J. CROZIER and E. S. HARRIS, Rutgers College.

Enumerations made of the animals inhabiting a sewage "filter," of intermittent sprinkling type, disclose an association of some 50 genera preadapted to this essentially artificial environment. Protozoans, nematodes, rotifers, and oligochaetes are the conspicuous animal constituents of an organized film upon the stones of the "filter," the density of the population being so great that in each (centrifuged) cubic centimeter of this material there are present an average of about 100,000 protozoans, 35,000 nematodes, 4,000 rotifers and 150 annelids.

Seasonal fluctuation and succession of these forms has been followed quantitatively by means of periodic sampling at four levels of the "filter," at intervals of about two weeks for a period of more than a year. To some extent variations in the fauna may be perhaps directly correlated with known environmental factors; other cyclic fluctuations arise rather as mechanical consequences of the self-regulating changes of the "film" as a whole. With reference both to the individual forms concerned and to the cyclic adjustment of the whole film which is an expression of their integrated activities, the results are important for the theory of adaptation applying to this large-scale experiment involving the peopling of a novel environment.

89. *Some myriapods of South Bass Island, Ohio.* STEPHEN R. WILLIAMS, Miami University.

For the past three years a single colony of *Pauropus huxleyi* (?) has been found by a log in a woods near the Ohio State Fish Hatchery, Put-in-Bay.

With the *Pauropus* a single individual of *Eurypaauropus spinosus* (Ryder) has been obtained. This extends the range of this species northward and westward from Gambier, Ohio, where it has been reported by Profesor Walton.

*Scutigerella immaculata*, usually very scarce, was in some abundance in the early part of the 1922 season.

Larval *Scytonotus* have been found for many summers but none mature enough to show sex organs.

In spite of the abundance of rocks, *Scutigera*, the house centipede has not been found on the island. It is on the peninsula of Marblehead ten miles away.

90. *American Opalinidae*. MAYNARD M. METCALF.

This paper lists the genera and species of Opalinids known from twelve regions of the western hemisphere which are somewhat demarcated zoögeographically; directs attention to the affinities of these species; and discusses briefly the significance of this affinities as indicating sources of origin and routes of spreading of the ancestors of the American Opalinids. The data have paleogeographic significance. Charts are used in illustration.

91. *The alternative color-phases of fishes*. (Lantern.) W. H. LONGLEY, Goucher College.

Characteristic changes in coloration are induced in many tropical fishes by changes in their relation to their environment, or by changes in its character, as they pass from place to place. The conditions under which alternative phases are displayed in a number of species will be illustrated. The obliterative effect of the coloration will be more clearly indicated than in pictures previously exhibited. A few slides without particular reference to the main thesis, but illustrating further the possibility of successful marine photography, will be shown.

92. *Further observations on the hydrogen-ion concentration of Chesapeake Bay water*.

R. P. COWLES, The Johns Hopkins University, and A. M. SCHWITALLA, St. Louis University.

The determination of the pH values for the Chesapeake Bay water has been continued, and forms a part of the procedure followed in a biological and hydrographic survey of that body of water for the U. S. Bureau of Fisheries. Observations have been made at a station near the center of the bay off the mouth of the Potomac River and this station has now been visited in June 1921, January 1922, and March 1922. On all three occasions the pH values have been determined from samples taken at the surface, 10 meters, 20 meters, 30 meters and the bottom at one and a half hour intervals for twenty-four hours.

In January there was almost no variation in the pH values from the surface to the bottom and little indication of diurnal variation. March observations showed a distinct decrease in pH values, i.e. a decrease in alkalinity, passing from the surface to the bottom and in general a decrease in pH values during the night and early morning. The June observations at all hours showed the largest decrease in pH value passing from the surface to the bottom a maximum of 8.1 at the surface and a minimum of 7.2 at the bottom. Diurnal variation was quite evident, especially at the lower depths.

93. *The bibliography of fishes*. E. W. GUDGER, American Museum of Natural History.

This work owes its inception and approaching completion to the vision, energy and enthusiasm of Dr. Bashford Dean and his associates. Volumes I and II, begun by Dr. Dean many years ago and carried to completion by the late Dr. C. R. Eastman, contain 36,000 titles of articles and books pertaining to fishes. Volume III, on which I have collaborated with Dr. Dean, contains an Addendum of 4400 titles

plus 2300 Pre-Linnæan titles, (i.e. *ante* 1758), various small sections of bibliographies, voyages, and travels, etc., an extended and highly analytical subject-catalogue, and an index to this latter.

The Subject-Catalogue, which is mainly the work of Mr. A. W. Henn, has its morphological and general part divided into 118 sections beginning with Abdominal Pores, and ending with Vision in Fishes. Here the vast literature of fishes is brought into logical order, identical subjects are grouped and surrounded by their likes. The second part of this Subject Catalogue contains the less analytical natural history subjects, distribution papers, the larger taxonomic papers, etc.,—i.e., those subjects wherein the species is the unit.

There will be an ordinary alphabetical index to the Subject Catalogue which will enable one to find not merely a large section, as Nervous System, but any subsection under this heading, as Optic Chiasma, Ependyma, Reissner's Fibre, etc.

The printing of the final parts may be expected by next summer and the completion of this work, which will bring to the student of fishes the whole literature on any minute and obscure subject, ought to be a tremendous factor in furthering the study of fishes.

94. *The effect of environmental conditions on the rate of development.* (Lantern.)  
A. O. WEESE, James Millikin University.

Cocoons of *Epeira gibberosa* (?), some of which were parasitized by *Arachnophaga picea* Riley were subjected to controlled atmospheric conditions, with results leading to the following conclusions:

1) High temperature and high relative humidity caused most rapid development of the spiders, but mortality was lowest under outdoor conditions, and spiders were unable to complete development or to emerge except under conditions of high humidity. 2) The parasites developed most rapidly when subjected first to freezing temperature, then to low temperature and low humidity, and finally to high temperature and high humidity. Mortality was greatest under outdoor conditions, and least when the cocoons were kept at high temperature and moderate or low humidity. 3) The threshold of development of the parasite was found to be lower than that of the host, but the latter developed more rapidly at high temperatures. 4) It was found possible to compute relative velocity factors for each set of conditions and to produce an approximate constant by their summation.

95. *Studies of animal aggregations: The temperature relation with isopods.* W. C. ALLEE, The University of Chicago.

Temperature is not the controlling factor in the formation of aggregations of either land or fresh water isopods under laboratory conditions, but does modify the tendency to form bunches.

With the fresh water isopod, *Asellus*, the general tendency to collect in closely knit bunches decreases with the passing of the breeding season and conditions which favor the formation of aggregations in April have little or no effect in late May.

Isopods placed in uniform temperature even at the height of the breeding season lose their tendency to form these aggregates in from one to five days. This reversal in the bunching reaction occurs more rapidly at higher than at lower temperatures. After such isopods have lost all tendency to form collections, either an increase or a decrease in temperature will restore the tendency.

In so far as they have been tested, similar results have been obtained with two species of land isopods and it is possible that some such temperature relation is of widespread occurrence among animals that may live more or less singly or may gather into flocks or other aggregations at certain seasons of the year.

96. *The tadpoles of the frogs of Okefinoke Swamp, Georgia.* A. H. WRIGHT and A. A. WRIGHT.

We have studied seventeen species in the swamp and in its outskirts. Eggs laid singly. *Hyla squirella*—bodies greenish, tail uniformly sprinkled with black dots; *Acris gryllus*—very long, black-tipped tails and projecting spiracular tube; *Pseudacris ocularis*—scattered distinct black dots on body, black band on musculature of tail, clear intervals between musculature and rims of the crests; *Hyla gratiosa*—beautiful green tadpoles, deep crests, young with black saddles on base of the tail, light line from eye to tail. Eggs laid in films. *Gastrophryne carolinensis*—black, flat tadpoles, neither mandibles, nor labia, nor labial teeth nor fringes, the spiracle median and just ahead of the anus; *Hyla cinerea*—greenish tadpoles, yellowish tails; *Hyla femoralis*—some red, and five bands on the tail; *Rana grylio*—with a black-banded bordered pinkish vinaceous band on tail, and a black line through middle of upper crest; *Rana sp.*—black-rimmed and black-banded tail, bluish venter, dorsum with greenish yellow flecks. Eggs in lumps. *Rana virgatipes*—material not yet studied; *R. aesopus*—tadpoles greenish, four series of spots on dorsum, tail with large spots; *R. sphencephala*—tadpoles like those of *R. pipiens*. Eggs in bands, later cylinders. *Scaphiopus holbrookii*—has bronzy tadpoles, translucent crests, rounded tail tip, oblique black bars on musculature; labial teeth 4/5. Eggs in files. *Bufo quercicus*—gray tadpoles, six or seven black saddles on musculature, heavily marked upper crest, venter one mass of color; *Bufo terrestris*—black tadpoles, upper crest less spotted, venter not one mass of color.

97. *A morphological mechanism in some instances of physico-chemical adaptation.* F. H. PIKE, Columbia University.

It has been argued that, since some instances of adaptation may be explained on a physico-chemical basis, they can not have arisen in organic evolution. It might be said further that natural selection can not have been operative in their presentation. A closer study of these cases is necessary before accepting such a conclusion.

The demonstration of the necessity for, and the actual participation in these physico-chemical processes of adaptation, of a structural or morphological mechanism which has definitely arisen in the course of organic evolution would change the complexion of this question. I have called attention to two such cases. In the reaction of a higher organism (dog) to variations in external temperature, the whole organism follows the principle of le Chatelier and Braun. A poikilothermal animal does not react in this way. (Rubner.) The reason for the difference in the reactions of the two types of animals to the same change in the external environment is to be sought in the development of a morphological mechanism in organic evolution. In my studies on the reactions of the central (nervous) respiratory mechanism, under varying conditions of blood supply, the application of the principle of le Chatelier and Braun has also been shown. Here also the reactions of the animal is dependent upon a morphological mechanism which has arisen in organic evolution.

## I. GENETICS SECTIONS

102. *The transmission of the polycladous character in Sphacrocarpos Donnellii.* CHARLES E. ALLEN, University of Wisconsin.

The 'polycladous' clones, derived from two aberrant male plants, probably of common origin, which appeared in a culture of *S. Donnellii*, are distinguished by: crowded, markedly irregular lateral lobes; antheridia few, often lacking in considerable regions, naked or subtended by lateral leaf-like, lacerate, or ciliate involucre; occasional cup-shaped or approximately tubular involucre, rarely approaching the typical form; numerous dorsal lobes and cilia, some of which may represent involucre.

Typical ♀ × polycladous ♂ gave:

	Typ.	Polycl.	Doubtful	Undet.
♀	45	32	1	
♂	28	21		
Sex undet. (died young)		5		5

Polycladous females have the vegetative characters of polycladous males; also dorsal columnar upgrowths, which may represent involucre; so far as dissection has shown, they lack archegonia, and attempts at fertilization have been uniformly without result. A few clones show rare involucre approaching the typical form, and one clone bears at times a considerable proportion of such involucre. Cytological study by Mr. A. M. Wolfson shows that they possess the chromosome group characteristic of typical females, the polycladous males having the chromosomes characteristic of typical males. The "doubtful female" is possibly an intermediate form.

A cross of a polycladous male with a tufted female ("tuftedness" being a variable character apparently inherited in different degrees by the offspring of a single cross) gave:

	Type. to Tufted (Not Polycl.)	Polycl.	Doubtful	Undet.
♀	16	10		1
♂	8	7	1	1 (?)
Sex undet. (died young)				10

The aberrancies characteristic of tufted races would be masked by those of the polycladous form; probably the tufted potentiality is inherited by some of the polycladous offspring.

103. *Color changes in maize pericarp and the nature of the gene.* WILLIAM H. EYSTER, University of Missouri.

This paper is concerned with a dilute red pericarp which changes to more dilute reds and colorless as well as to more intense reds. There are frequent changes also to a number of variegations which also are unstable. The variegations change to reds of different intensities and to colorless. Colorless types thus originated appear fixed, while the deep reds are fairly stable.

The gene for these color changes is one of a large series of multiple allelomorphs for pericarp color in maize. It is usually quite stable until late in ontogeny when it

becomes unstabilized and changes with surprising frequency. From the nature and frequency of the color changes it is concluded that the gene is a complex structure composed of opposing gene elements. When the gene loses its stability late in ontogeny like elements are mutually attracted, while unlike ones are repelled. Consequently mitotic divisions will occur which will partially or completely separate the opposing elements and a variegation will result.

Variegation in pericarp color, flower color, in chlorophyll distribution, and the like, is the result of a gene changing from unstable to stable conditions.

104. *Inheritance of a primitive sporophyte in maize.* WILLIAM H. EYSTER, University of Missouri.

In the Spermatophytes the fertilized egg develops immediately into a sporophyte which becomes dormant while still in an embryonic stage. Such an embryonic sporophyte with the surrounding tissues is called a seed. This paper is concerned with a maize sporophyte which fails to enter the dormant state, but continues its development uninterruptedly from the fertilized egg to the plant. This characteristic behaves like a simple mendelian recessive character, and is linked with a factor for pale yellow endosperm with about 1.25 per cent crossing over, and a factor for chlorophyll development.

105. *Notes on heritable endosperm defects of maize.* E. W. LINDSTROM, Iowa State College.

Two new types of heritable defects of corn endosperm have occurred over a period of three years. Three inbred generations of a commercial strain of Golden Bantam sweet corn afford conclusive data on the Mendelian inheritance of a simple, recessive endosperm-defect.

A similar result was obtained with a strain of Yellow Flint corn. This recessive defect, however, proved to be completely linked in inheritance with the pure white or albino seedling character.

106. *Eight years selection for quality of oil in soy beans.* L. J. COLE, E. W. LINDSTROM and C. M. WOODWORTH.

Beginning in 1912 with a single plant, high and low selection for drying quality of soy bean oil was practised for eight years. A significant separation of the two selection lines occurred, the high line showing a slight upward tendency and the low line a distinct downward trend. These results are interpreted as due to the isolation of different genotypes from an original mixture.

A distinct correlation between drying quality of oil and season of maturity was found. The high line proved to be distinctly later and of a different type of growth than the low line.

A slight negative correlation was found to exist between quality and percentage of oil. This was so small as to be of no commercial importance.

107. *The relation between chromosome number and morphological characters in wheat hybrids.* KARL SAX, Maine Agricultural Experiment Station.

A cross of *Triticum vulgare*, with 21 haploid chromosomes,  $\times$  *T. durum* with 14 haploid chromosomes resulted in partially sterile  $F_1$  plants and an  $F_2$  population

with all degrees of sterility. Chromosome counts of 46  $F_3$  plants showed 21 with 14 haploid chromosomes, 12 and 21 chromosomes and 13 plants with an intermediate chromosome number. In the  $F_2$  and  $F_3$  the individuals with intermediate chromosome numbers are rapidly eliminated due to sterility and it appears that the ultimate homozygous fertile segregates will have either 14 or 21 chromosomes. A high degree of correlation was found between chromosome number, morphological characters, and rust resistance. All of the  $F_3$  plants with 14 chromosomes had the distinguishing characters and rust resistance of the durum parent while all of the  $F_3$  plants with 21 haploid chromosomes had the morphological characters and susceptibility to rust of the vulgare parent. Cytological and genetic evidence indicates that the characters which distinguish the *vulgare* varieties from the *durum* and Enmer varieties are due to the 7 additional chromosomes of the vulgare varieties. The unpromising prospects of combining the desirable characters of the durum wheats with the high quality of gluten of the vulgare wheats are obvious.

111. *Anatomical differences between the various chromosome mutants of Datura.* E. W. SINNOTT and A. F. BLAKESLEE.

A study of the minute anatomy of the various chromosome mutants of *Datura* makes it evident that each of these mutants, heretofore recognizable only through external characters, is also readily distinguishable internally. The structure of the floral peduncle was found to be the most favorable region in which to observe these distinctions. Mutants differ markedly in number, width and depth of vascular bundles, in development of interfascicular cambium, in width of bast, in character of pericycle and in other ways. The addition of an extra chromosome in one of the pairs thus changes radically the whole internal and external structure of the plant. The so-called "acolytes" were found to show marked resemblances to their respective "apostles." The normal diploid plant is intermediate in its anatomical characters between the extremes shown by the mutants.

118. *Factors which determine otocephaly in guinea pigs.* SEWALL WRIGHT, U. S. Department of Agriculture.

Among some 40,000 guinea pigs in the genetical experiments of the Bureau of Animal Industry, 82 monsters of the otocephalic type have been recorded. They may be classified in a practically linear series of 12 grades of defectiveness, culminating in an almost headless condition. These grades may be interpreted in harmony with Child's gradient theory.

The majority (50) have appeared in one vigorous inbred family. There are marked differences among sables of this family. Several produce 1 to 4 per cent, one produces over 20 per cent.

While there are important genetic differences between different families and sub-families, the occurrence of the monsters can not be explained as due to Mendelian segregation or by mutation, except where one whole subfamily becomes differentiated in its tendency. Evidence indicates that the character is not maternal and that it is not a specific effect of inbreeding. Females are twice as likely to be affected as males.

There is a tendency, but a very slight one, for otocephali to be bred under unfavorable conditions; as indicated by month of birth, size of litter, weight and mortality

of littermates. The main factor must be one which affects the individual, not the litter.

By elimination it is concluded that the main factor is probably chance delay or other irregularity in implantation (as suggested by Mall) acting in conjunction with genetic factors which have a general influence on the metabolic level at a particular critical moment in development.

119. *Persistent new color patterns in grouse locusts by mutation, and linkage, or homozygosis, with isolation.* ROBERT K. NABOURS, Kansas Agricultural College.

There has been at least one mutation among the *Paratettix texanus* individuals reared in the laboratory. This one has bred true for seven years, through twenty-five generations, and numerous combinations from which it has invariably segregated according to Mendelian expectation. It has lately been placed in nature, and although not yet recovered, indications point to its survival as well as any others.

Numerous combinations of dominant patterns have been produced in *Apotettix eurycephalus* by linkage of factors in one series, or on one pair of chromosomes, and others by developing individuals (*P. texanus*) homozygous for two, or three patterns, the factors probably being located in different series, or on different pairs of chromosomes. It is possible, in fact, likely, that individuals homozygous for the patterns of these combinations might become isolated and persist till the normal (wild?) and elementary patterns, which would disrupt the combination, become extinct, at least in the adjacent territory.

120. *The consequences of different degrees of interference, in the crossing-over of the hereditary genes.* H. S. JENNINGS, Johns Hopkins University.

The paper gives the general results of a mathematical study of the relations exhibited in the so called crossing over of the genes, and compares these with those bound to occur if the genes are arranged in series and there is interference between breaks at points near together in the series. The observed ratios fall into a peculiar and complex system; by means of a mathematical formula it is shown that this entire system, even to details, is, in all essentials, a necessary result of the serial arrangement with a certain extent of interference. The marked changes that would result from different extents of interference are likewise shown.

121. *Coincidence of crossing over in Drosophila virilis.* ALEXANDER WEINSTEIN, Johns Hopkins University. (Introduced by H. S. Jennings.)

Crosses involving practically the entire length of the X chromosome in *Drosophila virilis* (over 92 units) indicate that coincidence is low for regions close together and that, as the intermediate distance becomes longer, coincidence first increases and then decreases. Coincidence is at a maximum (about 1.0) when the intermediate distance is about half the length of the chromosome. Therefore there is no interference between crossing over at the center of the chromosome and crossing over at either end; and, as might be expected, triple crossing over involving the center and both ends simultaneously is not interfered with, the coincidence being almost 1.0. As is to be expected from the greater genetic length of the *virilis* X chromosome, the total frequency of triple crossing over is greater than in *D. melanogaster*, and quadruple



crossing over (to the extent of about 0.2 per cent) occurs about as frequently as triple crossing over in *melanogaster*.

122. *Notes on the pigment cells in the eyes of Drosophila eye-color mutants.* O. A. JOHANNSEN, Cornell University.

Forms examined: Eosine miniature, eosin vermillion, pink, purple, ruby, sepia, tinged, vermillion, white, wild.

The eye structure, including cornea, pseudocone, retinulae, rhabdome and pigment cells, does not differ materially in the nine eye-color mutants from that of the wild fly *Drosophila melanogaster*. The pigment cells are present in all, though the pigment granules are wanting in white, and in the male of Eosin miniature, and but feebly represented in Eosin miniature, female, Eosin vermillion, pink and tinged. In all cases the most distinct coloring is found in the cells in the distal part of the post retinal fibre layer. In the more deeply colored eyes, purple, ruby, sepia, and wild, the proximal part of the accessory or basal pigment cells are, next to the post retinal cells, most deeply colored. The distribution of the pigment granules from the base of the pseudocone nearly to the basal membrane is rather uniform, though quite sparse in the paler colored mutants. Chemically, the eye color offers some interesting features.

123. *Analysis of "Contamination" in Habrobracon.* P. W. WHITING, University of Iowa.

In stock with defective veins,  $r_4$ , selection for eight generations has no effect. Percentage of defectives fluctuated about 95 and mean grade of defectives about 6.5. Defective males were mated to normal stock females and sons were bred parthenogenetically from their heterozygous daughters. A few of the most defective sons were again crossed to normal and this alternation of sexual and parthenogenetic generations continued to  $F_{23}$ . If defective differs from normal by one factor,  $d$ , the parthenogenetic segregating generations should consist genetically of one normal,  $D$ , to one defective,  $d$ , the latter showing 95 per cent defectives with mean grade 6.5. But defectives were only 85 per cent of one-half the total with 5.9 as mean grade. This "contamination" appeared in first segregating generation,  $F_2$ , and was not increased by thirteen subsequent crosses to the same normal stock. Pure defective stock isolated at the end of this series immediately reverted to original condition, showing "contamination" not permanent. Modifying factor,  $m$ , increasing percentage and grade of defectives was not lost for males with highest defect were selected for crossing from each segregating generation. Hypothesis is suggested that segregating generations consist of normal, 1  $DM$ , 1  $Dm$ , and genetically defective, 1  $dM$  (75 per cent somatically defective with mean grade, 5.3), 1  $dm$  (95 per cent somatically defective with mean grade 6.5). Attempt is now being made to derive theoretical stock,  $dM$ , showing 75 per cent defectives with mean grade, 5.3.

124. *Facts indicating abnormal fertilization in Habrobracon.* ANNA R. WHITING and P. W. WHITING.

When black-eyed males are crossed with orange-eyed females, orange males and black females are expected by sex-linkoid inheritance. A few black males, however,

appear. These patroclinous males represent seven-tenths of one per cent of the fertilized eggs that come to maturity. The remainder are females. From the reciprocal cross, orange male by black female, all normal offspring should be black. Patroclinous males in this case would be orange. Among 3824 black males and 2536 black females there was but one orange, a misshapen male which died as a pupa. Some patroclinous black males are weak, others die as naked pupae. Some appear to be retarded in development. Many, however, appear normal and mate readily. Mating tests divide these into three classes; the largest, sterile; a second slightly fertile; and a third of normal fertility. Daughters from second class tend to be morphologically defective and sterile, from third class normal in appearance and fertility. Patroclinous males transmit either their paternal or their maternal eye color, but in one case both black and orange daughters were produced from the same male. In normal stocks offspring, male and female, of mated females total from one-fourth to one-third fewer than offspring, male, of unmated females. These facts indicate that normal development is hindered by abnormal fertilization. The morphological defects and sterility of daughters of patroclinous males indicate injury to germ plasm.

125. *The birth rate among the graduates of Allegheny college.* H. R. HUNT, University of Mississippi.

Mental capacity is probably to a considerable extent inheritable. It is therefore important to determine whether the birth rate is tending toward a relative decrease in the number of intellectually superior persons. College graduates as a whole are probably inherently superior mentally to the population at large, yet recent investigations show that they produce relatively few children.

The following data were collected by questionnaires from the men and women who graduated from Allegheny College from 1870-1899. 316 (57 per cent) of the surviving graduates filled out the questionnaires. Of the 290 men, 11 per cent of those in the classes of 1870-1884 remained single, and 13 per cent in the classes of 1885-1899. The average sizes of completed families for these two periods are  $3.19 + .18$ , and  $2.83 + .13$  children, respectively. The difference,  $.36 + .22$ , is probably not significant.

The married men and women graduates, plus their consorts, and the unmarried graduates total 632. This group produced 752 children. Life insurance statistics show that only about 588 (93 per cent of 632) of these children will probably reach maturity (21 years). When families with wives less than 45 years old are excluded, and the number of unmarried persons is reduced in proportion, the 632 is diminished to 425. Of the 531 children of this group, 415 (98 per cent of 425) will probably survive at least 21 years. It is therefore probable that this collegiate group has not more than replaced itself.

126. *Sex-ratios in guinea-pigs.* (Lantern, 10 min.) HEMAN L. IBSEN and LUELLE SCHAUMBURG, Kansas State Agricultural College.

Out of a total number of 7989 guinea-pigs produced during the years 1913 to 1921 inclusive, 4128 were found to be males and 3861 females, making a sex-ratio of 106.92 (51.67 per cent) males to 100 (48.33 per cent) females.

Contrary to results reported by Papanicolaou (1921), no significant difference was found in the sex-ratios of animals belonging to litters of one size as compared

with those of other sizes. By similar methods it was determined that the sex-ratio for animals born in a particular month was not significantly different from that of animals born during any other month.

There seems to be no differential sex mortality. This is true of 320 still-born animals and of 485 others that died before they were 20 days of age. In neither case was the sex-ratio of the dead animals appreciably different from that of the complete litters from which these animals came.

When the age in months of the mothers at parturition was taken into consideration, some rather unexpected results were obtained. For instance, females 15 months of age gave birth to 145 males and 87 females, while females 8 months of age produced 192 males and 224 female offspring. The difference in percentage of males between these two cases is 6.06 times the probable error of their differences, i.e., if the above difference were entirely due to chance it would be likely to occur only once in 20,000 trials.

127. *An environmental factor causing variation in weight at birth of guinea-pigs.* (Lantern, 10 min.) HEMAN L. IBSEN, Kansas State Agricultural College.

In guinea-pigs the modal gestation period is 68 days and the average weight at birth approximately 85 gms. The live weight at birth ranges from 35 gms. to 145 gms. The average weight of foetuses from females pregnant 65 to 67 days depends upon the number of young in the uterus, as is shown by the following table:

Days in Uterus	Number of foetuses in uterus							Number of Litters
	1	2	3	4	5	6	7	
65	109.2	91.0	90.06	72.67	73.78	65.54	62.51	37
66		92.52	88.70	80.90	77.04	73.34		8
67			103.27	94.10				3

This table points to the possibility that crowding in the uterus tends to decrease the weight at birth. Other facts bear this out. It has been found that there is a fairly high correlation between the total distance on either side of a placenta and the weight of the placenta. For the 65 day stage the correlation coefficient is  $.4623 \pm .0634$ , and for the 66, 67, and 68 day stages combined it is  $.6137 \pm .0743$ . For the 65 day stage the correlation coefficient of weight of placenta and weight of attached foetus is  $.7578 \pm .0246$  and for the following three days combined it is  $.7764 \pm .0404$ . The weight of the placenta thus varies directly with the amount of space on either side of it, and at the same time the foetus weight is highly correlated with the weight of the placenta to which it is attached. It seems probable that the effect of crowding upon foetuses is indirect, influencing first the placenta and secondarily the foetuses.

128. *A brief description of abnormalities observed in the descendants of X-rayed mice.*

C. C. LITTLE, University of Maine, H. J. BAGG, Memorial Hospital, New York.

Among the F<sub>2</sub>-F<sub>2</sub> descendants of mice given a light dosage of X-rays, a large number of individuals of the following types have appeared.

1. General head abnormality (lethal) resulting in the single or combined appearance of the following characters: (a) Absence of all trace of one or both eyes, (b) Hypertrophy or under development of the tongue, (c) Reduction or absence of the

lower jaw or of the mouth opening, (d) General reduction in body size and vigor (50+ cases).

2. Eye abnormality: a dry, crusted, hemorrhagic lesion involving the iris or whole eye on one or both sides producing typically blindness, but in rare cases approaching and overlapping normal. (300+ cases).

3. Deformities of any or all feet or legs, in some cases involving brachydactyly, in others syndactyly, and in some both. Curvature and marked distortion is often found. (25+ cases).

4. Dry, hemorrhagic lesion of the type listed under (2) resulting in absence of cranium and in dead acraniate monsters (5+ cases).

5. Dry, hemorrhagic lesion over lumbar region of spine resembling roughly, spina bifida (1 case).

6. Shortened hair on the flanks and sides (where hooded rats are white) normal hair on anterior end of body and on mid-dorsal stripe down back (in region where hooded rats of Castle's Grade +2 or +2½ are pigmented. (10+ cases.)

129. *The inheritance of an eye-abnormality appearing among the descendants of X-rayed mice.* C. C. LITTLE, University of Maine, H. J. BAGG, Memorial Hospital, New York.

A pair of mice female 85 Brown and male 49 Brown were given one-fifth erythema dose of X-rays on five successive days. Among their F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub>, F<sub>6</sub>, and F<sub>7</sub>, descendants more than 300 abnormal young have appeared. The abnormality which in rare cases overlaps normal somatically is a dried, crusted, hemorrhagic lesion of one or both eyes. It is clearly a mendelizing character and is recessive to normal. Its inheritance has been tested not only by matings within the X-rayed lines but by an outcross and by its transmission through the male only.

130. *The inheritance of a lethal head abnormality appearing among the descendants of X-rayed mice.* C. C. LITTLE, University of Maine, H. J. BAGG, Memorial Hospital, New York.

A pair of mice female 84 Brown, and male 36 Brown, were given one-fifth erythema dose of X-Rays on five successive days. Among their F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub> descendants a considerable number of abnormal young have appeared.

In the abnormality studied, the characters affected may be the eyes (one or both) the mouth (jaws, tongue) and the general size and vitality. The abnormality is lethal, resulting in the death of a great majority of the animals possessing it at birth, or during the first day.

The abnormality appears in proportions indicating strongly that it is due to a mendelizing gene and is recessive in nature.

In crosses of DD × DR, or DD × DD, sixty-one normal, and no abnormal have been obtained.

In crosses of DR × DR, the numbers obtained are 54 normal, 19 abnormal, the expectation on a 3 : 1 basis would be 55 to 18.

The abnormality has now been inherited in this way to the F<sub>4</sub> generation from the mice which were given the X-ray exposure.

131. *A report of a histological study of the eyes and gonads of mice treated with a light dosage of X-rays.* L. H. SNYDER, Carnegie Institution, M. SCHNEIDER, Carnegie Institution, C. C. LITTLE, University of Maine.

Mice were given a dosage of X-rays similar to that given the ancestors of the abnormal mice referred to by Little and Bagg in papers given in this program, and the histological appearance of their eyes and gonads were studied. No differences between the treated and untreated animals were found, and the conclusion is therefore that the chances favor the interpretation that the inherited abnormalities above referred to are in the nature of a direct effect of the X-rays upon the hereditary material underlying the characters involved.

132. *The production of non-disjunction by X-rays and the hereditary transmission through untreated females of the XXY condition so produced.* JAMES W. MAYOR, Union College.

In a previous communication the writer described what he called at the time the "elimination" of the X chromosome by X-rays. Further investigation has shown that the X-rays produce non-disjunction by which exceptional females as well as exceptional males are produced. A considerable number of exceptional daughters have been obtained after submitting the mothers to X rays before mating. A large proportion of these exceptional daughters have been found to be fertile and have bred further exceptions without having been submitted to X rays. The data establishes that the exceptional condition (XXY) produced as a result of treating mothers with X rays is hereditarily transmitted. Equational non-disjunction has also been produced by X rays and the XXY condition of this case transmitted through untreated daughters. The experiments have shown that X rays may produce a specific modification of the hereditary mechanism which is inherited as such.

133. *The effects of X-rays on the albino rat.* FRANK BLAIR HANSON, Washington University.

One hundred and thirty rats, both males and females, were exposed for varying periods of time to the action of X-rays. Females in the later stages of pregnancy when given the proper dosage produce litters in which one or more of the young have serious eye defects, changes in the shape of the skull, and considerable retardation in growth in some instances. Nearly all such young prove to be sterile.

However, one female, treated in utero three days prior to its birth, showed marked eye defect of the right eye, skull small and somewhat abnormal in shape, general roughened appearance of the coat, and humping of the back-bone. This female was mated to a normal male and early produced a small litter which she was apparently unable to nurse, and as they were refused by foster mothers, died. At the present writing she has just thrown a litter of six young and is nursing them. As the eyes are unopened yet it is not known whether the defects have been transmitted. Several other such defective-eyed females are now half grown.

A brother of the female mentioned above had both eyes defective and paralysis of the hind limbs. His scrotum is of normal size, but the testes, by palpation, seem to be about the size of peas and are very hard nodules. He is completely sterile. Two other rats of this litter showed marked hemorrhage of the brain, and died soon after birth. This litter is typical of many.

134. *Effects of alcohol fumes on three generations of albinorats.* FRANK BLAIR HANSON.

Three generations of rats have been treated with alcohol fumes. The experiment began with two litters of twenty rats. Each litter was divided into control and treated groups. All matings are brother by sister within the litter. No eye defects or other gross abnormalities have been produced.

Treatment began at twenty days of age. Measurements of body weight, body length, and tail length were taken at ten day intervals. In the first two generations no apparent effects were produced except in fertility which is markedly lowered in the treated group. In the third generation there are quantitative differences between the treated and controls as follows:

1. *Body weight:* At twenty days the control male's mean weight was  $27.22 \pm 0.94$ ; the treated  $20.85 \pm 0.49$  grams. At the same age the control female's mean weight was  $26.73 \pm 1.13$ ; the treated  $21.60 \pm 0.78$ . At 100 days the control males weighed  $135.45 \pm 7.51$ ; the treated males  $104.70 \pm 3.60$ . At this age the control females had a mean weight of  $130.00 \pm 5.66$ ; the treated females  $79.00 \pm 6.64$ .

2. *Litter size:* The mean litter size of the controls is  $5.00 \pm 0.47$  animals per litter; of the treated  $8.38 \pm 0.44$ .

3. *Birth weight:* The mean birth weight of the controls was  $6.55 \pm 0.18$  grams; of the treated  $5.18 \pm 0.05$ .

4. *Sex ratio:* The sex ratio of the controls was 48 males to 37 females, a percentage of  $56.47 \pm 3.63$ . In the treated it was 69 males to 40 females, a percentage of  $63.30 \pm 3.12$ .

The experiment is being continued.

135. *Results of breeding potato beetles under changed environmental conditions.* A. W. BELLAMY, University of Chicago.

One of thirteen pairs of beetles, all of which first deposited eggs in the greenhouse (control) and later, from eggs deposited under experimental conditions, gave an  $F_2$  among which were one male and two females with broken elytral stripes. This character was kept through  $F_4$ .

Environmental factors condensed:

	Greenhouse (control)			Breeding Chamber		
	Max.	Min.	Average	Max.	Min.	Average
Temperature	38° C.	7	22	38.5	34.5	35.9
Rel. Humidity	100%	51	66	46	24	36
Rate of evap. cc. per hour	1.9	0.10	0.72	4.4	0.78	2.45

The entire stock of beetles is from one pair out of one of Prof. Tower's old stocks. Brother-sister matings were used throughout except for two matings, one in the fourth and one in the sixth generation, where a single male was mated to a female from nature (Urbana, Ill.). The experiment began in the seventh generation. Although several collateral lines produced a few broken stripe individuals in the third, fourth generations, no such individuals ever appeared in the direct line of descent, or elsewhere after the first out-cross, except those from the experiment. The material from which the Urbana females came was apparently free from the character. Collections on two successive years revealed no trace of it. The character *may* have been induced or "fixed" by the experimental conditions. It *may*

have been carried as a recessive although the out-crosses and brother-sister matings tend to lessen this possibility. Its appearance *may* have been coincident with the experiment. Attempts made in the third and fourth generations to "fix" the character were not successful.

*136. Inheritance of weight in poultry.* HENRY G. MAY, Rhode Island Agricultural Experiment Station.

Work begun by Dr. Philip Hadley at the Agricultural Experiment Station of Rhode Island State College and continued by the author since 1920 shows that after the first two months the White Cornish breed develops faster than the Silver Spangled Hamburg breed. At nine months the two breeds average respectively 2200 and 1350 grams in case of the males and 1600 and 1050 in case of the females. In the Cornish males, growth is continued thereafter more slowly for seven months, when a further more rapid increase brings the weight at twenty-one months to about 3000 grams. In the Hamburg males growth ceases at ten months and is resumed again at eighteen months, bringing the final weight at twenty-one months to 1750 grams. In the females growth ceases in both Cornish and Hamburgs at about ten months and is resumed again at eighteen months, producing a weight of 2400 and 1700 grams respectively at twenty-one months. All crossbred birds develop as rapidly as the Cornish or in some cases even more rapidly. They attain their maximum growth at eight to ten months, when they average slightly above the weight of the Cornish, thereafter they lose weight and do not again come up to the Cornish weight. At twenty-one months they are intermediate between Cornish and Hamburgs.

The crossbreds reared include first generation crosses in both directions and second generation crosses of three different types. The second generation crosses show no greater variability than do the Cornish or the first generation crosses.

*137. A lethal type in mice, which may live for a few days after birth.* (Lantern; 5 min.) J. A. DETLEFSEN, The Wistar Institute.

In strains of black-eyed white mice a type of young has occurred rather frequently, which invariably dies one to three days after birth. Their appearance is distinctive and they can always be identified, since they are about one-half normal size and present a very white and bloodless appearance. Their occurrence suggests that the homozygous black-eyed white (which is lethal as in the case of homozygous yellow) may perhaps develop in some instances far beyond the stage supposedly characteristic of the homozygous yellow.

*138. Linkage studies in mice.* (Lantern; 5 min.) J. A. DETLEFSEN and E. ROBERTS, The Wistar Institute and University of Illinois.

Crosses of dark-eyed black spotted waltzers with pink-eyed brown self-colored normals gave quadruple heterozygotes, which mated inter se produced 596  $F_2$  young. All four allelomorphic pairs proved to be independent. While the numbers are as large as in Darbishires cross, no evidence for the elimination of waltzers was found.

*139. The heredity of the "white hooded" albino rat.* DOROTHY R. STEWART. (Introduced by Frank Blair Hanson.)

In the course of some experiments with the albino rat it was evident that many of the animals were what we have called "white hooded albinos." When the "baby

fur' is shed—usually between the thirtieth and fiftieth days—it is by a process of differential shedding. The hair was lost first on the dorsal side in an area variable in extent, but corresponding in outline to the colored hooded pattern. The skin, showing through the thin hair, gives a pinkish cast to this region which persists until the new hair grows in. The hair on the rest of the body is shed while the new hair is appearing in the "hood," so that the pattern remains distinct for about two weeks. After that these rats are not distinguishable from ordinary albinos. Their breeding capacities are, however, quite different.

A white hooded albino female, answering the above description, when mated with a wild gray male gives all gray offspring. These, when crossed back with white hooded albinos, produce six classes of young: gray, black, gray hooded, and black hooded in apparently equal proportions, and also white hooded and pure albinos. Since a 1 : 1 ratio of gray and albino would ordinarily be expected of such a back cross, it would seem that our white hooded albinos carry the factor for self-coloration as a double recessive. Experiments are in progress to test the genetic constitution of these animals.

*140. Recent studies on the relation of metabolism to sex.* OSCAR RIDDLE, Carnegie Station for Experimental Evolution.

The suprarenals of pigeons undergo marked hypertrophy coincident with each ovulation period. The enlargement persists over a total of 7 to 11 days. The reproductive overwork shown by Whitman and by the author to result in a changed sex ratio is therefore accompanied by an almost continuous change in this important metabolic organ. That the enlargement of the suprarenal medulla is accompanied by an increase of function has been fully demonstrated by a study of the blood sugar curve in relation to ovulation. The blood sugar value rises to 20 per cent above normal and its persistent high value clearly coincides with the period of suprarenal enlargement. These results hold for various species and hybrids.

That a low storage value (small size) of the ovum of the pigeon indicates a high rate of metabolism in such an ovum has been shown by measurements of the size of ova produced under the administration of glandular products whose action on the basal metabolism is definitely known. The secretion of the thyroid is known greatly to increase the metabolism and probably exercises this power not through intermediary mechanisms but directly by raising the oxidizing power of the cells. Ova produced under treatment with thyroid substance become markedly and consistently smaller. The thymus gland is known to exert no influence on the gaseous exchange; its administration has been shown to leave the storage metabolism of the ova unchanged. Studies with still other substances further confirm these results.

*141. Further studies of the rate of mutation in the bar series of Drosophila.* CHARLES ZELENY, University of Illinois.

The attempt to locate factors affecting the rate of mutation from bar to full has been unsuccessful so far but the existence of differences in rate has been demonstrated.

In the data previously presented before the society there were 52 mutants among 85,008 individuals, a rate of 61 per hundred thousand. One of two new series has essentially the same rate. The exact figures are 22 in 38,387 or a rate of 57 per hundred thousand. Another series however has a markedly lower rate with only



29 mutants among 104,019 individuals or 28 per hundred thousand. The difference is probably significant but the factors involved have not yet been analyzed.

Another interesting fact concerns the relative numbers of females and males among the mutants. In both of the new series the proportion is strikingly different from that of the old series. In the old series there were 31 females and 20 males while in New Series A there are 11 females and 18 males and in New Series B, 10 females and 12 males or a total for the two new series of 21 females and 30 males. It was concluded from the data of the old series, as reinforced by other considerations, that 31 females to 20 males indicates an approach to a two to one ratio which is to be expected on the assumption that female and male sex cells are equally subject to mutation. The data of the new series are obviously in conflict with this view.

142. *The measurement of mutation frequency made practicable.* H. J. MULLER, University of Texas, Austin, Texas.

The method of measuring mutation frequency by counting the mutant genes accumulated in chromosomes protected from natural selection by balanced lethals (Muller, 1920) has various advantages over the separate testing of X-chromosomes each generation (Muller and Altenburg, 1919), but still involves excessive labor for (1) the several hundred crosses and selections establishing all the lines free from unknown mutant factors, (2) mere propagation of all cultures throughout the accumulation process, (3) the several hundred elaborate crosses for finally testing all lines,—some doubtful mutants, moreover, require further testing, of stocks derived from rare combinations; failure to obtain these may invalidate the entire experiment.

The following synthetic stocks overcome difficulties (1) and (3)

$$\text{"A": } \frac{T^d b c_n c a m_r}{C_y c_n^2 C_{cr}} \frac{\Delta}{H}, \quad \text{"B": } \frac{P}{C_y c_n^2 C_{cr} C_{III}} \frac{\Delta H T_r}{l_{im}.$$

To start the lines (1) merely place one A male and B females in each vial. All progeny are killed genetically except the required, self-perpetuating  $\frac{T^d b c_n c a m_r}{C_y c_n^2 C_{cr}}$ .

In later generations, homozygous  $T^d b c_n c a m_r$  (female sterile) also appear, provided no unknown lethal existed at the start; cultures having such homozygotes (easily identifiable through the bottle) absent or abnormal are discarded. For final tests (3) one male from each line, preferably A type, is crossed to B. Inspection of  $F_2$  bottle reveals whether a new lethal has killed  $T^d b c_n c a m_r$  homozygotes; all  $F_2$  constitute stock for further testing.

The propagation difficulty (2) is overcome by embedding vials in concrete blocks, covered with cotton. For transferring, the flies are anaesthetized within the blocks, then uncovered, and shaken *en masse* into other blocks.

A new field of genetic research is thus opened up.

143. *Sex-linked inheritance in the teleost *Platypoecilus maculatus* Günth.* A. W. BELLAMY, The University of Chicago.

In connection with other work on these and allied fishes some evidence has been obtained indicating that four color phases occurring in *Platypoecilus* are ex-linked multiple allelomorphs, with the female the heterogametic sex. Nigra (N), rubra (R), and pulchra (P) are dominant to white (W). Probably any two of the four

characters will act as an allelomorphic pair although a number of crosses are not completed.

(1) A homozygous  $N\sigma \times W\varphi$  gave in  $F_1$ , 9  $N\sigma$ , 6  $N\varphi$  and in  $F_2$ , (2)—4 matings—98  $N\sigma$ , 68  $N\varphi$ , 65  $W\varphi$ , 1  $W\sigma$ . (3)  $F_2 N\sigma \times F_2 N\varphi$  gave—3 matings—32  $N\sigma$ , 31  $N\varphi$ . Another (4) gave 13  $N\sigma$ , 6  $N\varphi$ , 6  $W\varphi$ . These data are in agreement with the hypothesis that (1) is  $NN \times WO$ ; (2)  $NW \times NO$ ; (3)  $NN \times NO$ ; (4)  $NW \times NO$ . The remaining matings are given with the supposed gametic constitution. (5)  $RW \times RO$  gave (2 matings) 30  $RR$  and  $RW$ , 25  $RO$  12  $WO$ . (6)  $RW \times RO$  (father  $\times F_1$  daughter) gave  $RR$  and  $RW$  28,  $RO$  45,  $WO$  31. (7)  $RW \times NO$  gave  $RN$  2,  $WN$  5,  $RO$  7,  $WO$  4. (8)  $RN \times WO$  from (7) gave  $NW$  and  $NO$  11,  $RW$  and  $RO$  13—fish too young to determine sex. (9)  $NN \times RO$  gave  $NR$  2,  $NO$  2. (10)  $NR \times NO$  from (9) gave  $NR$  7  $NN$  4,  $NO$  7,  $RO$  6 and in addition there are 10 immature  $N$ , 6 immature  $R$ . The data for *pulchra* are similar to those given for *rubra* and *nigra*.

Four exceptions have occurred. 1  $W\sigma$  in (2) and 3  $W\sigma$  in a mating from (6), presumably  $RW \times RO$  which gave 12  $RR$ , and  $RW$ , 6  $RO$ , 11  $WO$  and 3  $W\sigma$ .

In the above formulae the "O" is used to designate the (supposedly) heterogametic female. Nothing is known concerning the chromosome relations. These data disagree in several important respects with the findings of Aida for *Aplocheilus latipes* (Genetics 6: 554-573) and Winge for *Lebistes reticulatus* (Jour. Genetics 12: 145-162).

144. *Further control of sex in a species of Cladocera.* ARTHUR M. BANTA and L. A. BROWN, Station for Experimental Evolution.

Experiments have demonstrated that the simple expedient of crowding the mothers causes the production of a considerable percentage of males among the offspring of several species of Cladocera.

This paper considers data for *Moina macrocopa*. Ten to 100 per cent males are produced in bottles containing 10 mothers while mothers in individual culture bottles (with rare exceptions) produce only females in first broods. It was found that the critical period, during which sex may be controlled by environmental influences, is near the close of the third instar, just before the eggs are laid. This is known to be, for certain Cladocera at least, the time at which the single maturation division gets under way.

The percentage of males produced by mothers reared in crowded bottles may be reduced by any of several methods of aeration. This suggested scarcity of oxygen or accumulation of carbon dioxide as possible factors involved in sex control. But in uncrowded bottles neither decreasing oxygen (by bubbling nitrogen), nor directly increasing the carbon dioxide, nor excluding air from the surface of the water induced the production of males.

Further work is being done on the supposition that the specific factor involved may be the accumulation of an excretory product, other than carbon dioxide.

The control of the production of the sexual egg is accomplished by crowding young mothers with reduced food or by crowding in old culture water.

145. *A new mutation in Daphnia longispina and its inheritance.* ARTHUR M. BANTA, Station for Experimental Evolution.

This mutation is a conspicuous emargination or excavation of the normally almost

straight ventral head margin. It appeared in a single parthenogenetic individual, none of whose sisters possessed the character or produced young showing it.

"Excavated head" is inherited. All the descendants of the original mutant genetically possess the character, but it varies greatly in its somatic manifestation. Half or more than half the individuals show no morphological evidence of the character; a considerable percentage have it only slightly or moderately developed; and only a very few show the extreme development of the character, in which the entire ventral head region between the long slender beak and the eye is deeply excavated. All these classes of individuals,—the somatically 'normal,' 'slight,' 'intermediate,' and 'extreme' produce the excavated head in approximately the same percentages of their offspring. Further, the apparently normal mothers produce as high grade excavated heads as mothers showing extreme development of the character.

In spite of the peculiarities of its manifestation, excavated head behaves genetically quite as one would expect a character to behave in a parthenogenetic stock in which there is no segregation in the maturation of the egg.

#### LATE ABSTRACTS

On account of complications growing in the main, out of intersociety relations, the following abstracts were received too late to be included among those originally printed. They are given here in order to present a complete report of the proceedings of the meeting. The number preceding each abstract is the serial number which the title carries in the list of titles.

42. *Notes on the entrance of the spermatozoon into the starfish egg.* ROBERT CHAMBERS, Department of Anatomy, Cornell University Medical College, New York City.

Eggs in a sperm suspension apparently accelerate the movements of the spermatozoon without attracting them.

The glutinous jelly surrounding the eggs entraps the spermatozoa which accidentally strike it. As the spermatozoa are unable to pierce the jelly they remain stuck to its surface.

The presence of the spermatozoa causes the surface of the egg to produce nipple-like elevations. From the summit of each nipple a filament grows out through the jelly. Its tip fuses with the motionless head of a spermatozoon. It then retracts dragging the spermatozoon into the eggs.

The retraction of this filament is accompanied by the lifting of a membrane from the surface of the nipple. This membrane elevation spreads over the egg to produce the fertilization membrane. The retraction of the filament is also accompanied by a retraction of all other filaments. More than one filament may secure a spermatozoon, and polyspermy may then result. The breaking up of delayed filaments and the spread of the fertilization membrane assist in preventing polyspermy.

A certain minimum concentration of the sperm suspension is necessary to cause the egg to respond. This response is a function of the original egg cortex whether it be a fragment or entire. This explains the non-fertilizability of endoplasmic spheres.

The cortex of immature eggs behaves like that of mature eggs. The presence of neither the jelly nor of the egg membrane is necessary for fertilization.

43. *The subdivisions of the neural folds in man.* G. W. BARTELMEZ, The University of Chicago.

This analysis of the nervous system is based upon the recognition of certain landmarks: viz., 1) the otic segment which is distinctive in form, in the behavior of the neural crest arising from it and which is definitely related to other landmarks like the otic plate; 2) the midbrain which is located by the cranial flexure; 3) the first hindbrain segment, characterized by the behavior of its neural crest; 4) the first pair of somites.

In a two-somite embryo there are five enlargements of the neural folds: forebrain, midbrain, and three hindbrain segments. The first and last in the hindbrain subsequently divide, but the middle segment, the otic, remains distinct throughout the series and in the 4-mm. stage it is obviously the fourth rhombomere of the usual enumeration. One of the primary subdivisions of the folds is accordingly a definitive neuromere. The other subdivisions elongate and then neuromeres are separated from them. The differentiation thus evidenced begins caudally and proceeds forward. First the postotic ('rhombomere 5') and then the preotic ('rhombomere 3') appears; then the sixth and seventh are differentiated. Before this the first and second separate, but there is some doubt as to whether the 'first rhombomere' is a true neuromere. After the rhombomeres are formed the already pending division of the midbrain into two typical neuromeres is completed. Still later the primary forebrain divides.

86. *Reactions of Hydra to chloretone.* WM. A. KEPNER and D. L. HOPKINS, University of Virginia.

In reacting to chloretone the ectoderm behaves as a neuromuscular tissue; while the endoderm functions as a neuroid tissue.

A sphincter has been observed at the base of each tentacle. This sphincter operates in preventing material from passing from the enteron into the tentacle, but does not prevent passage of material from the tentacle into the enteron.

There is no extensive diffusion of absorbed chloretone through the tissues of the body. A diblastic animal, therefore, cannot possess anything comparable to a circulatory medium.

Green hydras under ordinary conditions withstand the effects of chloretone much more than do brown and gray ones. Our experiments indicate that the presence of zoochlorellae of *Hydra viridis* is responsible for this hydra's resistance to chloretone, for we can greatly lower this resistance by placing the green hydras in the dark. This lends weight to the contention that the algae of green hydras endoderm are symbionts.

87. *Blood and nerve as controlling agents in the movements of melanophores.* LELAND C. WYMAN. (Introduced by G. H. Parker.)

By making a vertical incision through a part of the tail of a specimen of *Fundulus heteroclitus* L., the pigment motor nerves to the melanophores in the region posterior to the incision are severed and the distal portions of these nerves degenerate in a

few days. The blood supply to this region remains normal. The melanophores in the denervated area thus produced expand at first, but in four or five hours they partially contract, assuming a stellate form and remain in that condition until acted on by a direct stimulus. These denervated melanophores can be used as indicators to determine whether chemical substances introduced into the body of a fundulus at a point anterior to the incision have their action directly on the pigment cells being carried to them by the blood or have an indirect action through the nervous system. By this method it is found that a number of anesthetics and alkaloids when applied to the gills or injected into the body cavity cause various responses of the melanophores due to the action of the chemicals on some part of the nervous mechanism. Direct application of the same substances to the denervated melanophores causes similar responses, unless the chemical is one which rapidly coagulates the protoplasm of the cells. Alcohol, ergot, and several endocrin secretions, when applied to the animal in the same way, act directly on the melanophores, being carried to them in the blood. Salts have no indirect effect on melanophores. Although there is a close relation between the melanophores and the sympathetic nervous system, the cells can react normally when freed from nervous influences. Direct control by hormones or other chemical substances in the environment is an important adjunct to nervous control.

98. *Caddisfly larvae of swift and standing waters.* G. S. DODDS, West Virginia University, F. L. HISAW, Kansas Agricultural College.

Forty-one species of caddisfly larvae from lakes and streams of the Rocky Mountains in Colorado form the basis of this study. These bodies of water range from stagnant ponds to the swiftest mountain torrents and have a rich population of mayfly nymphs and caddisfly larvae, both of which have been successful in populating almost all parts of both ponds and streams. Special attention is given to the problem of locomotion and retention of position in the swift streams. The caddisfly larvae, on account of the presence of the protective case, built by the insect itself, have, of necessity, met these conditions in a different way from the Mayfly nymphs and other animals without such a case. The present paper is an analysis of the habitats and types of cases found in each, and involves such points as habits of larvae, selection of materials, architectural instincts, difference in size, form and structure, and the species without cases.

99. *An octo-flagellate parasitic in trout.* EMMELINE MOORE, New York State Conservation Commission, E. S. A.

Octomitiasis is an intestinal disease of fish produced by the protozoan, *Octomitus*, an organism widespread in this country and endemic in many trout-rearing hatcheries. The infection is traceable to wild or domesticated fish which having survived the disease become carriers of the organism. Under conditions producing epidemics, the disease becomes rapidly fatal. Sexual and asexual cycles occur in the host with encystment stages in the epithelial tissue of the intestinal tract. Culture material gives important information in the life-history studies.

108. *Induction of chromosomal mutants and their recognition in Datura.* ALBERT F. BLAKESLEE and M. E. FARNHAM.

Balanced chromosomal types in *Datura* already known are 1n, 2n, 3n, 4n. Tetra-

ploid (4n) plants have been obtained in two ways:—a) from selecting, among 2n seeds, the rare 4n seeds recognized by their large size; b) from treating 2n parents with cold. Triploid (3n) offspring may be obtained by crossing a 4n with a 2n plant. The full range of (2n + 1) mutants may be expected from crossing a 3n with a 2n plant. Haploid (1n) plants have been induced by treating diploids (2n) with cold.

The balanced types are recognized by shape and size of leaves, flowers, capsules, etc.; their unbalanced types by the modifying effects of specific individual chromosomes. Pollen inspection is of aid in classifying balanced types in early stages. The even-balanced types 2n and 4n have relatively good pollen, but pollen grains of 4n plants is distinctly larger. The odd-balanced types 1n and 3n have a high percentage of bad grains; the few full grains from 1n plants are of the size of normal grains in diploids (2n), whereas the full grains from 3n plants show a great diversity in size.

109. *Induction of gene and chromosome mutations in Datura by exposure to radium rays.* C. STUART GAGER and A. F. BLAKESLEE.

Ovaries in young flower buds of *Datura stramonium* of stock inbred for about twelve generations were exposed to rays from radium emanation in a sealed glass tube, strength 13 microcuries, for ten minutes. This treatment was followed by a great increase in number of mutants, with maximum of 33.33 per cent for ovary cell nearest radium, 11.54 per cent for cell farthest away, and average of 16.96 per cent for all four cells. Average percentage of mutants in same stock for four years (1919–1922) was 1.38, with maximum in one case (1920) of 7.07 per cent, due possibly to low temperature of late fall.

110. *The distribution of chromosomes in tetraploid daturas.* J. BELLING and A. F. BLAKESLEE, Station for Experimental Evolution, Cold Spring Harbor, Long Island, New York.

Six size classes of chromosomes recognizable at all stages. Attraction of homologous chromosomes produces quadrivalents. Non-disjunction, leading to the formation of 23- and 25-chromosome pollen, occurs in from a quarter to a third of the reduction divisions. Some 47- and 49-chromosome progeny therefore occurs. Double opposed non-disjunction produces plants with 48 chromosomes, having ten quadrivalents, one trivalent, and one quinquivalent. Their progeny shows many 47- and 49-chromosome plants. Detachment of chromosomes. Non-reduction. Chromosomes of functional egg cells shown in cross of tetraploid by diploid. Tetraploid inheritance. Plants with a reduction or deficient chromosome produced by the cross of triploid by tetraploid. Twenty-four-chromosome pollen in diploid plants. Conclusion.

112. *Ratios of globe mutants to normal plants in Datura after pollination with counted pollen.* J. T. BUCHHOLZ, and A. F. BLAKESLEE.

Small amounts of pollen from globes and normal plants were counted and applied to the stigmas of both globes and normal daturas. The results obtained from these, compared with controls, indicates that the resulting small capsules contain on an average a much smaller proportion of globes than the well-pollinated large ones. In the smaller seed capsules, the proportion of aborted ovules found scattered among the

seeds is greater than in large capsules, and it is nearly always much greater in the lower half of any capsule than in the upper half. In globe  $\times$  normal crosses, both in full pollinations and in restricted pollinations, the lower half of the seed capsules usually contained a much smaller proportion of globes than the upper half. This difference was not so great and more irregular in globes than were sibbed or selfed. Any increase which may have been obtained in the number of  $(n + 1)$  pollen tubes entering the ovary in the restricted pollinations was more than offset by the selective elimination among the ovules of the smaller seed capsules. That the  $(n + 1)$  pollen tubes growing more slowly than those with  $n$  chromosomes is indicated by the fact that with only one exception there are more globes in the lower half than in the upper of the capsules from the normal  $\times$  globe crosses; also the only  $(2n + 2)$  globes obtained were from the lower half of the globe  $\times$  globe capsules. Data are from eight or more capsules in each cross.

113. *Species in the genus Rubus and Crataegus.* ALBERT E. LONGLEY.

Interested in the problems centered around imperfect pollen, I collected in the spring of 1921 flower buds of many species of *Rubus* and *Crataegus*. This material I have studied carefully in regard to the development of the pollen tetrad.

Recent work of Taeckholm and others on the genus *Rosa*, led to the apparently well-founded conclusions that many of the species in this genus are of hybrid origin.

I have come to a like result regarding many species of *Rubus* and *Crataegus*, based on the following phenomena, which one recognizes as characteristic of pollen development in hybrids.

1. Polyploidy, which is present in three-quarters of the *Rubi* examined.
2. Irregularities in the heterotypic and homotypic divisions, e.g., chromosomes that are tardy in arriving at, and leaving the plate.
3. The presence of ejected chromatin material in the cytoplasm, resulting from chromosomes failing to be included in the daughter nuclei.
4. The presence of dwarf nuclei, derived from chromosomes left in the cytoplasm.
5. Multinucleated pollen grains, due to the presence of dwarf nuclei.

114. *On a gigantic natural hybrid of the silverweed (Potentilla anserina).* E. C. JEFFREY.

The true silverweed, *Potentilla anserina*, s. str., occurs in salt marshes. A variety of it growing at the head of beaches and in grassy spots not much permeated by sodium chloride is known to systematic botanists as *Potentilla anserina*, var. *grandis*. The author has observed that this variety of silverweed is extremely sterile, only in the rarest instances setting any seed at all, and these always few in number. The pollen grains are largely aborted, and in this feature present a marked contrast to the true *P. anserina*. The cytology of the development of the gonads furnishes conclusive evidence of the hybrid character of this gigantic form of the silver-weed.

115. *On the origin of the Boston fern.* E. C. JEFFREY.

From the investigation of a large number of varieties of the Boston fern, as well as the original type, the conclusion is reached that this popular horticultural monstrosity is a hybrid. The cytological investigation has shown that the sporogeny of the Boston fern is strikingly unlike that of other and normal species of *Nephrolepis*

grown in the same greenhouse, and very similar to that of interspecific and bigeneric hybrid ferns. The conclusion is reached that the Boston fern is a hybrid, and consequently cannot be used as an example of mutation.

116. *Further evidence of crossing over in Oenothera.* GEORGE H. SHULL.

Notwithstanding the fact that students of *Oenothera* cytology find no situation which seems favorable to the occurrence of crossing over by the chiasmatype method generally advocated by the *Drosophila* students, the genetical evidence for the occurrence of crossing over in the *Oenotheras* is unequivocal.

Crossover data are here presented for (a) revolute leaves and red hypanthia (less than one per cent); (b) revolute leaves and red stems (2.45 to 3.08 per cent); (c) red hypanthia and sulfur colored flowers (6.1 to 8.7 per cent); and (d) sulfur-colored flowers and dwarfness (4.7 and 9.2 per cent). In some of the crosses the action of the two sets of balanced lethals,—the gamete lethals and the zygote lethals—are clearly manifested, and there is also some distortions of ratios by differential elimination. In one case there is an apparent release from the typical linkage relation, so that a typical dihybrid ratio is presented.

117. *A preliminary account of the genetics of Clarkia elegans.* L. L. BURLINGAME.

This paper describes the inheritance and factorial basis of the following color types: white, cerise, magenta, lilac, lavender and salmon pink. Two striking form variations of the plants are also described. Cerise and magenta are, respectively, due to a single mutation in two separate chromosome pairs. The double recessive is white and the double dominant is lilac. Lavender is due to a dominant mutation in the same chromosome as the magenta factor and is accordingly linked with cerise from which it crosses over in about 20 per cent of the gametes. A twining habit and a conspicuous open habit of growth are both recessive to the normal upright richly branched habit.

146. *The translocation of a section of chromosome-II upon chromosome-III in Drosophila.* C. B. BRIDGES, Carnegie Institution of Washington and Columbia University. (Introduced by T. H. Morgan.)

A mutant eye-color, Pale, was found to be a non-sex-linked specific modifier of eosin without effect by itself. Pale is a dominant with special relation to viability. A Pale by Star Dichaete male back-cross showed that Pale is produced by (besides eosin) a second-chromosome dominant ( $P_{II}$ ) and a third-chromosome dominant ( $P_{III}$ ). In heterozygous condition  $P_{II}$  is a zygotic lethal unless  $P_{III}$  is also present. In homozygous condition  $P_{II}$  is lethal even when  $P_{III}$  is present. Heterozygous  $P_{III}$  is slightly below par in viability when  $P_{II}$  is not present, but has normal viability when  $P_{II}$  is present in heterozygous form.  $P_{III}$  is completely lethal when homozygous, unless  $P_{II}$  is present in heterozygous form, in which case it is viable, but below par.

Crossing-over experiments show that the location of  $P_{II}$  is the entire section, of some eight units' length, from arc to the right end of the second-chromosome, throughout which region crossing-over is entirely suppressed.  $P_{III}$  is located to the left of rough and suppresses nearly all the crossing-over in the adjacent region,



viz., from spineless to the right end of chromosome-III. When  $P_{III}$  is present, all the recessive mutants (viz., plexus, brown, speck, morulla, balloon, blistered, purple-oid, and lethal-IIa) whose loci lie to the right of arc in chromosome-II fail to show as characters even when homozygous.

The above situation (with other factors not referred to) led to the interpretation that  $P_{III}$  is a broken-off end of the second-chromosome, and that  $P_{II}$  is the second chromosome from which this end has been broken.



# AMERICAN SOCIETY OF ZOÖLOGISTS

## CONSTITUTION, OFFICERS AND LIST OF MEMBERS OF THE SOCIETY

### CONSTITUTION

#### ARTICLE I

##### NAME AND OBJECT

*Section 1.* The Society shall be called the "American Society of Zoölogists."

*Sec. 2.* The object of the Society shall be the association of workers in the field of Zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical investigation in this country.

#### ARTICLE II

##### MEMBERSHIP

*Section 1.* Members of the Society shall be elected from persons who are active workers in the field of Zoölogy and who have contributed to the advancement of that science.

Zoölogists without the training in research required for full membership may be elected associate members of the Society.

*Sec. 2.* Election to membership in the Society shall be upon recommendation of the Executive Committee.

*Sec. 3.* Each member shall pay to the Treasurer an annual assessment as determined by the Society. This assessment shall be considered due at the annual meeting and the name of any member two years in arrears for annual assessments shall be erased from the list of members of the Society, and no such person shall be restored to membership unless his arrearages shall have been paid or he shall have been re-elected.

*Sec. 4.* Foreign Zoölogists, not members of this Society, may be elected Honorary Fellows upon unanimous recommendation of the Executive Committee by a majority vote of the members present at any meeting of the Society. Honorary Fellows shall not be required to pay dues.

#### ARTICLE III

##### OFFICERS

*Section 1.* The officers of the Society shall be a President, a Vice-President, a Secretary and a Treasurer and the members at large of the Executive Committee.

*Sec. 2.* The Executive Committee shall consist of the President, the Vice President, the Secretary, the Treasurer and five members elected from the Society at

large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve out the remainder of his term.

*Sec. 3.* These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

*Sec. 4.* The officers named in Section 1 shall discharge the duties usually assigned to their respective offices.

*Sec. 5.* Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled by appointment, until the next annual meeting, by the Executive Committee.

*Sec. 6.* At the annual meeting the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than one month before the next annual meeting. It shall be the duty of the Secretary to mail the list of nominations to all members of the Society at least two weeks before the annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

#### ARTICLE IV

##### MEETINGS OF THE SOCIETY

*Section 1.* Unless previously determined by the Society the time and place of the annual meeting of the Society shall be determined by its Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

*Sec. 2.* Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

#### ARTICLE V

##### QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of its Executive Committee.

#### ARTICLE VI

##### CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society by a two-thirds vote of the members present, upon the following conditions:

(a) The proposed amendment must be in writing and signed by at least five members of the Society.

(b) This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

(c) The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

## BY-LAWS

### DUES

(1) The annual dues for members or associate members, unless remitted or changed by the vote of the Society, shall be seven dollars.

### SECRETARY

(2) The duties and privileges of the Secretary shall be as follows:

(a) He shall keep the records of the Society.

(b) Whenever the proper officers of a number of related societies shall have a conference with a view to determining a common time and place for the several annual meetings, he shall act as the delegate or representative of this Society. (See also 5.)

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society, and

(d) He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

### TREASURER

(3) The duties and privileges of the Treasurer shall be as follows:

(a) He shall be in charge of the funds of the Society.

(b) At the Annual Business Meeting of the Society he shall present a statement to date of the funds of the Society.

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society.

### AUDITING COMMITTEE

(4) The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

(5) The National Research Council allows the Society three representatives on the Division of Biology and Agriculture. Of these three representatives, one shall be elected each year to serve three years. The method of election shall be the same as that used in the election of the officers of the Society.

### MEETINGS

(6) It shall be the policy of the Society to hold meetings in both Eastern and Central-Western territory, and the distribution of the meetings between the two territories shall be determined in general on the basis of the representation of Eastern and Western members in the Society. See also 2-b.

## PROGRAM RULES

(7) In matters relating to programs for annual meetings the following rules shall be observed:

(a) Papers shall be listed and presented according to subject matter in the following groups: 1. Comparative Anatomy; 2. Embryology; 3. Cytology; 4. Genetics; 5. Comparative and General Physiology; 6. Ecology, and 7. Miscellaneous, or other groups at the discretion of the Secretary.

(b) Whenever conditions require it the Executive Committee shall schedule two or more groups for the same hour and rearrange the program to bring together papers on subjects of more general interest for meetings of the whole Society. The Committee, however, is instructed to avoid conflicts as much as possible.

(c) Papers shall be listed in their respective groups in the order received. When a member offers more than one paper those following the one designated first shall be placed at the end of the list and shall not be read until all first papers by members shall have been twice called for.

(d) All papers not read when called for as listed shall be placed at the end of the group list, and, if not read when called for the second time, they shall be read by title only.

(e) The titles of "introduced" papers shall be listed in the groups after the titles of papers to be read by members. Such papers shall be read by title only in case the entire program cannot be completed during four regular sessions for reading papers.

(f) Fifteen minutes shall be the maximum time allowed for the presentation of a paper.

(g) Abstracts of papers for publication in the proceedings of the Society must be handed to the Secretary or his representative before final adjournment of the annual meeting.

(8) Associate members may become members of sections of the Society and are entitled to the journal privileges of members.

## HISTORICAL REVIEW

A review of the historical antecedents of the present American Society of Zoölogists will be found in *The Anatomical Record* for January, 1917. The list of officers and meeting places of the present Society found in the same place is brought up to date and reprinted here.

## LIST OF FORMER OFFICERS

## AMERICAN MORPHOLOGICAL SOCIETY

<i>President</i>	<i>Vice-President</i>	<i>Secretary-Treasurer</i>
1890—E. B. Wilson	.....	J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. S. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

*Additional Members of the Executive Committee*

1891—E. B. Wilson	1897—J. S. Kingsley
H. F. Osborn	Bashford Dean
1892—E. L. Mark	1898—C. B. Davenport
T. H. Morgan	F. R. Lillie
1893—T. H. Morgan	1899—J. P. McMurrich
C. B. Davenport	G. H. Parker
1894—E. A. Andrews	1900—F. R. Lillie
F. H. Herrick	Jacob Reighard
1895—T. H. Morgan	1901—C. F. W. McClure
S. Watase	C. W. Hargitt
1896—E. G. Conklin	1902—H. S. Jennings
William Patten	R. G. Harrison

## AMERICAN SOCIETY OF ZOOLOGISTS

EASTERN BRANCH	<i>President</i>	CENTRAL BRANCH
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
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W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward

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William Patten  
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*Vice-President*

1903  
1904  
1905  
1906  
1907  
1908  
1909  
1910  
1911  
1912  
1913

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H. F. Nachtrieb  
S. J. Holmes  
William A. Locy  
George Lefevre  
H. B. Ward  
M. F. Guyer  
M. F. Guyer  
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R. H. Walcott  
C. M. Child  
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G. A. Drew  
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H. S. Pratt  
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L. L. Woodruff  
L. L. Woodruff  
H. W. Rand  
Raymond Pearl  
J. H. Gerould  
Caswell Grave

1903  
1904  
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1913

Frank Smith  
F. R. Lillie  
C. E. McClung  
T. G. Lee  
T. G. Lee  
T. G. Lee  
Charles Zeleny  
H. V. Neal  
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W. C. Curtis  
W. C. Curtis

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H. S. Jennings  
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D. H. Tennent  
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H. E. Jordan  
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1914. C. E. McClung	M. F. Guyer	H. E. Jordan—1 year H. F. Nachtrieb—2 years H. V. Wilson—3 years George Lefevre—4 years A. F. Shull—5 years
1915. W. A. Locy	W. E. Ritter	D. H. Tennent
1916. D. H. Tennent	Charles Zeleny	R. P. Bigelow—5 years L. J. Cole—4 years
1917. M. M. Metcalf	Charles Zeleny	H. V. Wilson
1918. George Lefevre	L. L. Woodruff	M. M. Metcalf
1919. C. M. Child	H. H. Wilder	George Lefevre
1920. Gilman A. Drew	Caswell Grave	C. M. Child
1921. Charles A. Kofoid	Aaron L. Treadwell	G. A. Drew
1922. H. H. Wilder	Bennet M. Allen	C. A. Kofoid

*Secretary-Treasurer*

1914-1918. Caswell Grave  
1918-1921. W. C. Allee

*Secretary*

1921. W. C. Allee

*Treasurer*

1921. D. H. Tennent

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National Research Council*

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*Associate Editors of the Journal of Morphology*

1921

Gary N. Calkins      J. S. Kingsley      William Patten

1921-22

E. G. Conklin      M. F. Guyer      W. M. Wheeler

LIST OF PLACES OF MEETING

AMERICAN MORPHOLOGICAL SOCIETY

1890—Boston	1894—Baltimore	1899—New Haven
1891—Philadelphia	1895—Philadelphia	1900—Baltimore
1892—Princeton	1896—Boston	1901—Chicago
1893—New Haven	1897—Ithaca	1902—Washington
	1898—New York	

CENTRAL NATURALISTS

1899—Chicago      1900—Chicago

## SOCIETY OF AMERICAN ZOÖLOGISTS

1901--Chicago

1902--Washington

## AMERICAN SOCIETY OF ZOÖLOGISTS

## EASTERN BRANCH

1903--Philadelphia  
 1904--Philadelphia  
 1906--New York  
 1907--New Haven  
 1909--Boston  
 1910--Ithaca

## JOINT MEETINGS

1905--Ann Arbor  
 1908--Baltimore  
 1911--Princeton  
 1912--Cleveland  
 1913--Philadelphia

## CENTRAL BRANCH

1903--St. Louis  
 1905--(Mch.) Chicago  
 1907--(Mch.) Madison  
 1907--Chicago  
 1910--(Apr.) Iowa City  
 1910--Minneapolis  
 1912--(Apr.) Urbana

## MEETING PLACES

1914--Philadelphia  
 1915--Columbus  
 1916--New York

1917--Minneapolis  
 1918--Baltimore  
 1919--St. Louis

1920--Chicago  
 1921--Toronto  
 1922--Boston

# AMERICAN SOCIETY OF ZOÖLOGISTS

## OFFICERS AND LIST OF MEMBERS<sup>1</sup>

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<i>President</i> .....	M. F. GUYER
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<i>Secretary</i> .....	W. C. ALLEE
<i>Treasurer</i> .....	D. H. TENNENT

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GEORGE LEFEVRE .....	1923
C. M. CHILD .....	1924
GILMAN A. DREW .....	1925
CHARLES A. KOFOID .....	1926
H. H. WILDER .....	1927

### *Representatives of the Society in the Division of Biology and Agriculture of the National Research Council*

F. R. LILLIE .....	1923
H. S. JENNINGS .....	1924
E. G. CONKLIN .....	1925

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<sup>1</sup> The data given in this list is based on the last preceding list published in *The Anatomical Record*, Vol. 23, No. 1, with such corrections and additions as have come to the attention of the Secretary. Please notify the Secretary of errors in this copy of the membership list that they may be corrected in the next published list.

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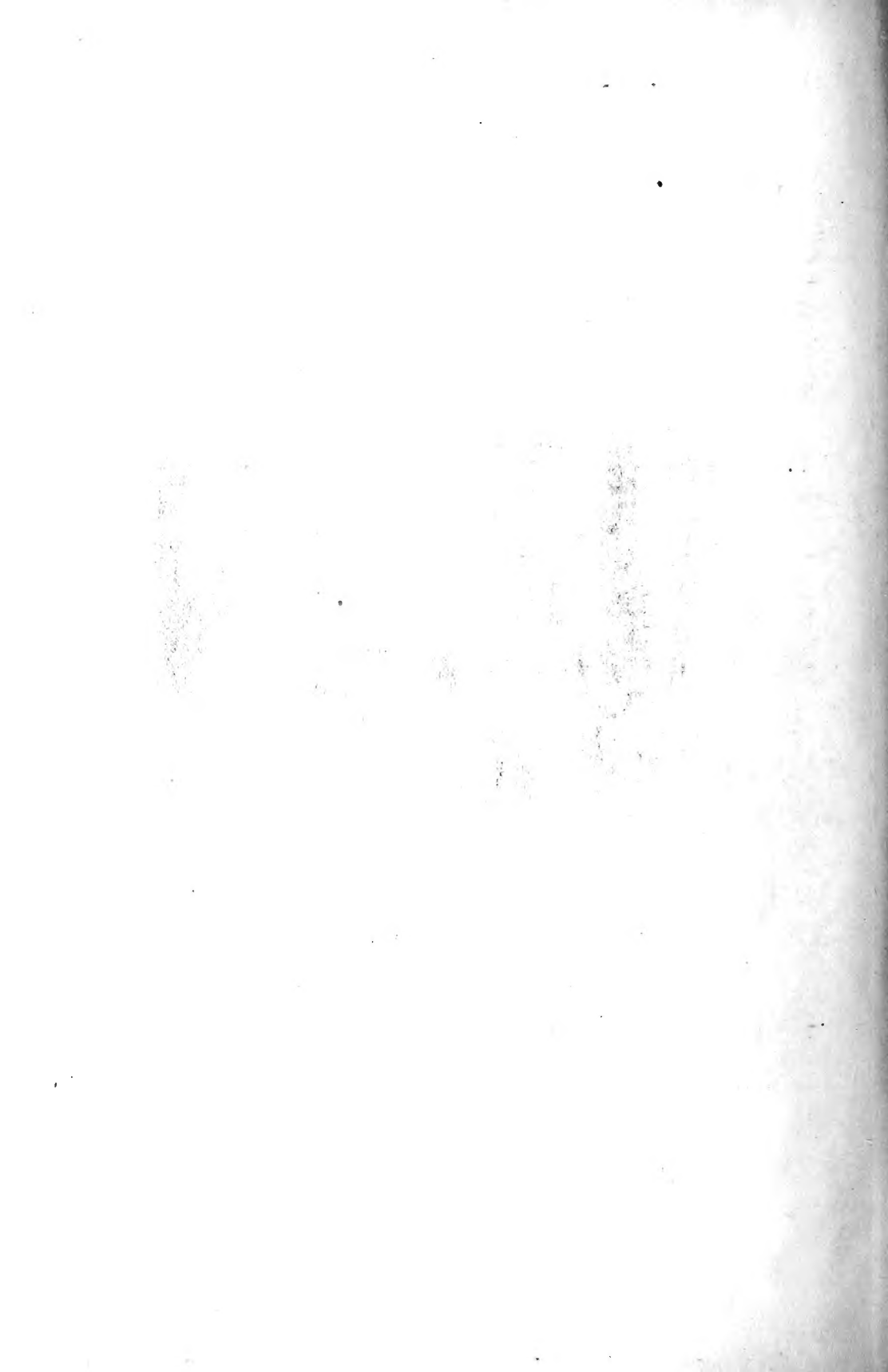












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